Predicting and Preventing Aspiration Pneumonia in Patients with Acute Stroke and Dysphagia

A Thesis Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Preface

This thesis conforms to the referencing style recommended by the American Psychological Association Publication Manual (6th ed.) and spelling recommended by the Oxford English Dictionary. The research presented in this thesis was conducted between November 2012 and April 2016 at hospitals within the Canterbury District Health Board, the New Zealand Brain Research Institute and The UC Rose Centre for Stroke Recovery and Research in Christchurch, New Zealand as well as the School of Dentistry at Otago University in Dunedin, New Zealand. Financial support was provided by the Sir Don Beaven Doctoral Scholarship as well as a Neurological Foundation of New Zealand Small Project Grant (E6153) and the Ministry of Health Oral Health Research Fund (E6171).

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Abstract

Patients with dysphagia following acute stroke are at increased risk of developing aspiration pneumonia, which leads to increased morbidity and mortality. Silent aspiration is a leading cause of aspiration pneumonia, but is notoriously difficult to identify by standard clinical swallowing examination. Adjunctive tests, such as cough reflex testing, have been evaluated as a means of increasing sensitivity of clinical assessment.

Historically, diurnal variation in cough sensitivity has been considered a major confounding variable that is controlled for by testing at the same time each day. Study I investigated whether such variation exists when the tidal-breathing method – commonly used in clinical assessment – is used to measure cough reflex sensitivity. Fifty-three young, healthy participants underwent cough reflex threshold testing on two occasions: once in the morning (between 9am – 12pm) and once in the afternoon (between 2 – 5pm). Oral bacteria levels were controlled for by participants' brushing their teeth immediately prior to testing. There was no evidence of diurnal variability in cough reflex sensitivity, with morning cough thresholds ($\tilde{\chi}$ = 0.4 mol/L) not significantly different from afternoon cough thresholds ($\tilde{\chi}$ = 0.4 mol/L), $p > .05$. Regardless of time of day, first cough thresholds ($\tilde{\chi}$ = 0.3 mol/L) were lower than second cough thresholds ($\tilde{\chi}$ = 0.5 mol/L), $p < .01$, raising the question of reproducibility of cough reflex testing; an issue which warrants further investigation.

Previous reports evaluating the effectiveness of cough reflex in acute clinical settings have provided mixed results. This has prompted careful consideration of how cough reflex testing should be translated into clinical routine and the development of a standardised management pathway. In Study II, pneumonia-related outcomes were

measured from 284 patients with acute stroke who were managed according to a Dysphagia in Stroke Protocol (DiSP). The DiSP guided clinical decision-making regarding oral intake based on clinical assessment with cough reflex testing and videofluoroscopic swallowing study (VFSS). Outcomes were compared to an historical cohort of 148 patients who were managed with cough reflex testing and VFSS in the absence of a prescriptive protocol. The rate of aspiration pneumonia was significantly lower among protocol-managed patients (10 %) compared to control patients (28 %). The odds of developing aspiration pneumonia were 3.24 times higher if no protocol was used. Although no differences in mortality were observed between the DiSP and control groups, patients in the DiSP group were more likely to be referred for a VFSS, spent significantly fewer days in hospital (24 days versus 32 days; $p < .001$) and were more likely to be on a normal diet at three months post-stroke compared to control patients. These data support the use of cough reflex testing when incorporated into a dysphagia management protocol.

Patients with acute stroke and dysphagia are also at increased risk for colonisation by potential respiratory pathogens, which, if aspirated, have been linked to the development of aspiration pneumonia. There is evidence to support a relationship between oral hygiene and reflexive cough sensitivity in the elderly, but this relationship is poorly understood. To characterise this relationship, 102 patients with acute stroke and dysphagia underwent saliva sampling and cough reflex threshold testing at three time points: on admission to hospital, at discharge from the acute stroke ward and at one month post-stroke. In addition, patients' medical notes were reviewed for symptoms of aspiration pneumonia. Molecular testing was used to measure the prevalence of pathogenic bacteria in saliva samples. No relationships between cough reflex sensitivity, oral bacteria levels and aspiration pneumonia were

found; however, results were limited by low statistical power. A non-significant trend for bacteria levels to increase following admission to hospital was observed, which warrants further investigation.

This programme of research has contributed to the field of speech-language therapy by providing the first report of the effectiveness of the DiSP in reducing the incidence of aspiration pneumonia among patients with acute post-stroke dysphagia, as well as identifying future research directions for studies of oral bacteria in this population. Finally, this research has contributed to the growing body of knowledge regarding cough reflex testing methodology.

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Abbreviations

ANOVA = analysis of variance

AP = aspiration pneumonia

ASU = Acute Stroke Unit

BLAST = Basic Local Alignment Search Tool

CAP = community-acquired pneumonia

Cq = quantitation cycle

CDHB = Canterbury District Health Board

CPH = Christchurch Public Hospital

CRT = cough reflex testing

CRTT = cough reflex threshold testing

CVA = cerebrovascular accident

DHB = District Health Board

DiSP = dysphagia in stroke protocol

dNTP = deoxynucleoside triphosphate

ERS = European Respiratory Council

gDNA = genomic DNA

HCAP = health-care-associated pneumonia

ICU = intensive care unit

LER = laryngeal expiratory reflex

LOS = length of stay

MDT = multidisciplinary team

MRSA = *Methicillin-resistant Staphylococcus aureus*

NA = nucleus ambiguus

NBM/NPO = nil by mouth/*nil per orem*

NGT = nasogastric tube

NTS = nucleus tractus solitarius

NVP = non-ventilator ICU pneumonia

NZ = New Zealand

OR = odds ratio

PCR = polymerase chain reaction

PEG = percutaneous endoscopic gastrostomy

qPCR = quantitative polymerase chain reaction

RCT = randomised controlled trial

SCT = suppressed cough threshold

SLT = speech-language therapist; speech-language therapy

SOHS = specialised oral hygiene service

spp. = several bacterial species within one genus

TAE = Tris-acetate-EDTA

TBI = traumatic brain injury

UC = usual care

VAP = ventilator-associated pneumonia

VEES = videoendoscopic evaluation of swallowing

VFSS = videofluoroscopic swallowing study

Part A: Introduction and Literature Review

Chapter 1. Introduction

Dysphagia represents a significant healthcare issue as it is associated with increased morbidity, mortality and associated healthcare costs. In the acute post-stroke period, patients are at increased risk of complications related specifically to aspiration pneumonia (AP). Multiple risk factors for AP have been identified, including silent aspiration and the aspiration – and lack of clearance – of pathogenic oral bacteria into the lungs. This research programme focused on predicting and preventing AP in an acute stroke population based on these risk factors.

Part A provides a review of the literature including an overview of swallowing physiology, reflexive airway protection mechanisms and oral bacteria. The consequences of dysphagia are discussed with particular attention to the acute post-stroke period as well as the strengths and limitations of current clinical assessment and management approaches. The role of the reflexive cough response in airway protection is described as well as the consequences of impairments to this reflex (dystussia). Current issues in cough reflex testing methodology are also discussed. Finally, the role of oral bacteria in the pathogenesis of AP is reviewed with emphasis on previous research findings and evidence supporting a link between oral bacteria and cough reflex sensitivity is presented.

In Part B, three studies are presented that are related to predicting and preventing AP among patients with acute stroke and dysphagia. In Study I, attention was given to cough reflex testing methodology. Specifically, the question 'does diurnal variation exist in cough reflex testing?' was asked. Study II investigated the effects of the Dysphagia in Stroke Protocol in an acute patient population. Study III examined the relationships between pathogenic oral bacteria, cough reflex sensitivity and AP among patients with acute post-stroke dysphagia.

In Part C, the results of the research programme are discussed. Due to the translational nature of this research, results have direct implications for clinicians working in acute dysphagia management. The DiSP has already been translated into current clinical practice in a number of hospitals, but continued refinement of the protocol to incorporate other risk factors for pneumonia is recommended. The impact of methodological limitations on the results is discussed and directions for future research are identified, particularly with regard to further studies of oral bacteria in patients with acute stroke. This research programme has contributed to the advancement of speech-language therapy practice by addressing gaps in the knowledge base supporting the use of cough reflex testing in clinical care and has also opened the door to future collaborative research between the fields of microbiology, respiratory medicine and speech-language therapy.

Chapter 2. Normal Swallowing

2.1 Swallowing Physiology

Swallowing is a complex biological process that facilitates the transport of food from the oral cavity through the oesophagus and into the stomach in a safe and timely manner. While in reality swallowing is a single dynamic event, in order to conceptualise this, it can help to describe swallowing in distinct functional phases.

Daniels and Huckabee (2008) describe swallowing as a four-stage process. The first phase is the pre-oral phase, in which preparation for the incoming bolus takes place. At a mechanical level, this may include the initiation of airway protection mechanisms (e.g. vocal fold closure) and increased salivation. At a higher cortical level, this may involve the monitoring of bolus size and rate of ingestion, as well as associating the visual and olfactory aspects of the bolus with certain memories. The second phase is the oral phase, where bolus acceptance, manipulation, and mastication occur, facilitated by volitional tongue movement. Bolus transfer from the oral cavity into the pharynx marks the end of this phase, and the beginning of the third (pharyngeal) phase of swallowing. This phase is characterised by a rapid succession of events: hyolaryngeal excursion, velopharyngeal closure, base of tongue to posterior pharyngeal wall approximation, pharyngeal shortening, epiglottic deflection, laryngeal valving, and relaxation of the upper oesophageal sphincter. During this phase, breathing must also cease to facilitate airway protection, and then rapidly resume. Temporal coordination of these components is crucial for the safe and efficient transfer of the bolus through the pharynx (Daniels & Huckabee, 2008). The final phase is the oesophageal phase, during which peristaltic contractions from the striated muscles and sequential contractions from the smooth muscles of the

oesophagus propel the bolus through the upper and lower oesophageal sphincters and into the stomach.

This system is not without potential for complications due to the anatomy of the swallowing mechanism. Due to the close proximity of the oesophagus and trachea, the potential for airway compromise is significant. In a healthy system, this risk is minimised by intrinsic pulmonary protective mechanisms.

2.1.1. Pulmonary protective mechanisms.

Due to the importance of pulmonary protection for survival, multiple airway protective mechanisms exist. During swallowing, the hyoid bone and larynx move anterosuperiorly and there is concomitant deflection of the epiglottis and pharyngeal shortening. The true and false vocal folds adduct upon activation of the thyroarytenoid, cricothyroid, lateral cricoarytenoid and vocalis muscles, providing another layer of airway protection. Compression of the quadrangular membrane effectively seals the entrance to the larynx during swallowing. This anatomical configuration serves to prevent material from entering the laryngeal vestibule – known as penetration – and proceeding into the tracheal airway – known as aspiration.

Another mechanism of pulmonary protection is the cough response. There are different types of cough response, including the cough reflex and the expiratory (or laryngeal adductor) reflex. The cough reflex is defined as “forced expulsive manoeuvre against a closed glottis” (Morice et al., 2007, p. 1256) whereas the expiratory reflex is defined as “a forced expiratory effort (without preceding inspiration) with closure of the glottis, followed by opening of the glottis and an expulsive phase” (Widdicombe & Fontana, 2006, p. 10). Although both reflexes have distinct neurological afferents and are subject to different modulations and inhibitors (Widdicombe & Fontana, 2006), the terms are often used interchangeably. In reality,

the expiratory reflex often occurs in conjunction with a series of coughs and the distinction between the two types of reflex may not be clinically relevant (Addington et al., 2003).

2.2. Neural Control of Swallowing

Swallowing is mediated by a complex neural network involving cortical and subcortical regions of the brain that relay sensorimotor information to the brainstem. It is accepted that the basic motor plan for swallowing is produced by a central pattern generator in the medulla (Daniels & Huckabee, 2008). This central pattern generator is comprised of groups of nuclei, including the nucleus tractus solitarius (NTS) and nucleus ambiguus (NA).

The bilateral NTS, located in the dorsal region of the medulla, are the primary sensory nuclei for cranial nerves VII, IX, and X, and thus receives afferent signals from the tongue, pharynx and larynx, including the superior laryngeal nerve (critical for initiating the reflexive cough response). Cranial nerve V, with bilateral sensory nuclei located in the pons, also provide afferent information to the NTS. Important sensory information from the mucosal receptor cells in the pharynx, muscle spindle receptors in the pharyngeal and oesophageal muscles and specific cortical regions, converge in the NTS to facilitate the programming of a motor output for swallowing (Daniels & Huckabee, 2008). This contributes to the precision of muscle contraction (e.g. timing, pressure) that is required to swallow a variety of different bolus sizes, textures, and temperatures (Daniels & Huckabee, 2008).

The second key components of swallowing motor control are the bilateral NA, located in the ventral medulla. The NA are the primary motor nuclei for cranial nerves XI, X and XI and thus supply input to the soft palate, pharynx and larynx, among other structures. Importantly, the NA receives direct efferent input from the NTS. This

connection allows the specific motor plan for swallowing, generated in the NTS, to be received by the NA and transmitted to the pharynx, larynx and oesophagus. Of particular interest is the presence of direct connections from the superior laryngeal nerve, essential for the elicitation of the protective cough reflex, in both the NTS and NA. In the case of a reflexive cough, bypassing the NTS and generating this response directly in the NA may be a survival adaptation in response to an aspiration event.

Animal studies using repetitive electrical brain stimulation have identified that supratentorial regions of the brain directly influence swallowing (e.g. Miller & Bowman, 1977; Sumi, 1969). By using neuroimaging techniques in human subjects, researchers have identified a number of cortical structures that are activated during swallowing, including the pre- and post-central gyri, superior temporal gyri, middle and inferior frontal gyri, supplementary motor area, parietal cortex, anterior cingulate cortex, insula, frontal operculum and caudal anterior cingulate cortex (Huckabee, Deecke, Cannito, & Gould, 2003; Martin, Goodyear, Gati, & Menon, 2001; Martin et al., 2004; Suntrup et al., 2013; Vasant et al., 2014). However, in neural activation studies it is difficult to distinguish between cortical areas that are responsible for swallowing-associated functions (e.g. jaw movement) and swallowing-exclusive brain regions (Malandraki, Sutton, Perlman, Karampinos, & Conway, 2009). Studies which use blood oxygen level measures are also unable to differentiate excitatory versus inhibitory activation (Leopold & Daniels, 2009). The implication of the research to date is that *any* brain lesion has the potential to modify or disrupt swallowing in some form. In other words, disordered swallowing is not purely a function of a brainstem or bilateral cerebral insult.

2.3. Summary

Swallowing is a complex physiological process that involves timely and precise coordination of over 50 paired muscles innervated by five cranial nerves. While heavily brainstem-modulated, it also relies on input from cortical and subcortical areas. Stroke is a permanent brain injury caused by a sudden interruption in blood flow to the brain and can, as a result, impair swallowing.

Chapter 3. Stroke-related Dysphagia

3.1. Cerebrovascular Accident.

Cerebrovascular accident (CVA), also known as stroke, is a permanent loss of brain tissue as a result of disrupted blood flow. The vast majority of strokes occur as a result of an ischaemic event, where a thrombus (blood clot) or embolus (arterial plaque) becomes lodged in a blood vessel, disrupting the flow of blood. Eighty percent of strokes are ischaemic in nature (Della-Morte et al., 2012). The remaining twenty percent are attributable to a haemorrhage caused either by a ruptured blood vessel or a cerebral arteriovenous malformation (Birenbaum, 2010). In New Zealand, stroke represents the third largest cause of death (Ministry of Health, 2009). The long-term consequences of stroke depend upon the site and severity of the lesion and the speed and effectiveness of the treatment.

Dysphagia, or disordered swallowing, is a common consequence of acute stroke arising from injury to the neural, sensory, and/or motor systems that underlie deglutition. Medical literature suggests that the incidence of dysphagia is particularly high in stroke patients. Between 25-81 % of patients experience dysphagia in the acute stages of stroke, with the wide variation in incidence due to poor agreement between sources regarding what constitutes the acute phase and differing definitions of dysphagia (Daniels & Foundas, 1999; Gottlieb, Kipnis, Sister, Vardi, & Brill, 1996; Kidd, Lawson, Nesbitt, & MacMahon, 1993; Langdon, Lee, & Binns, 2007; Mann, Hankey, & Cameron, 1999; Marik & Kaplan, 2003; Martino et al., 2005; Meng, Wang, & Lien, 2000). For many patients, swallowing function eventually resolves, however, persisting dysphagia has been documented in 50 % of stroke patients after 6 months (Mann et al., 1999).

The consequences of post-stroke dysphagia are often debilitating and can hinder recovery, resulting in increased length of hospital stay and long-term care requirements (Odderson, Keaton, & McKenna, 1995). Addressing outcomes for stroke patients with dysphagia has become a key focus of acute stroke care worldwide.

3.2. Pathophysiology

Stroke-related dysphagia is highly variable in presentation between patients, depending, in part, on the type of stroke and lesion laterality. Patients with haemorrhagic stroke are more likely to experience dysphagia compared to patients with ischaemic stroke (Paciaroni et al., 2004). However, the vast majority of strokes are ischaemic (Wardlaw, Murray, Berge, & del Zoppo, 2009). Lesions of the right hemisphere tend to be associated with pharyngeal dysfunction (e.g. poor pharyngeal motility, increased risk of aspiration) whereas left hemisphere lesions tend to result in oral dysfunction (e.g. poor bolus manipulation and transport [Robbins, Levine, Maser, Rosenbek, & Kempster, 1993; Robbins & Levine, 1988]). Although Daniels & Foundas (1999) reported no inter-hemispheric differences in dysphagia presentation, their dysphagia rating scale relied heavily upon observed penetration/aspiration, which is not a necessary feature of dysphagia. It has also been suggested that there may be a "dominant swallowing hemisphere" (Hamdy et al., 1997; Singh & Hamdy, 2006) which, if damaged, is more likely to result in dysphagia compared to a lesion in the non-dominant hemisphere (Hamdy et al., 1997). Others, however, have reported more significant dysphagia (i.e. aspiration) in patients with lesions to the non-dominant hemisphere (Robbins & Levine, 1988).

Specific site of lesion also plays a significant role in dysphagic presentation. Bilateral cranial nerve damage is more likely to result in dysphagia and aspiration

compared to unilateral lesions (Horner, Massey, & Brazer, 1990; Horner & Massey, 1988). Anterior lesions (relative to the central sulcus) are more often associated with the presence of dysphagia and aspiration, compared to posterior cortical lesions (Daniels & Foundas, 1999; Robbins et al., 1993). Lesions of the precentral gyrus tend to affect the contralateral facial, lip, tongue, and pharyngeal muscles (Veis & Logemann, 1985). Large vessel lesions (e.g. middle cerebral artery) are more strongly associated with aspiration compared to smaller vessel lesions (e.g. deep white matter [Daniels & Huckabee, 2008]). Brainstem strokes, while not as common as cortical strokes, tend to impair swallowing function the most (Martino et al., 2005). Lesions in this area can alter oral sensation and impair coordination of pharyngeal swallowing (i.e. the precise sequence of events involving swallowing initiation, hyolaryngeal excursion, airway closure, and upper oesophageal opening [Martino, Terrault, Ezerzer, Mikulis, & Diamant, 2001]). Other sites that have been associated with dysphagia include the pre-motor and primary motor cortices, primary somatosensory cortex, insula and periventricular white matter (Alberts, Horner, Gray, & Brazer, 1992; Daniels & Foundas, 1999; Daniels, Foundas, Iglesia, & Sullivan, 1996).

Even if the stroke occurs in an area of the brain that is not directly associated with swallowing, dysphagia may still be present. This is due to normal, age-related changes in anatomy, sensation and cognition. Symptoms such as prolonged bolus transport time through the oropharynx, decreased upper oesophageal sphincter (UES) resting tone and opening and increased post-swallow residue have all been documented in healthy, ageing populations (McKee, Johnston, McBride, & Primrose, 1998; Rademaker, Pauloski, Colangelo, & Logemann, 1998). Since the incidence of stroke increases with age (Devroey, Van Casteren, & Buntinx, 2003), these normal swallowing changes may exacerbate dysphagia caused by stroke (Martino et al.,

2005). Conversely, an elderly post-stroke patient may have reduced capacity to compensate for age-related swallowing changes (Martino et al., 2005).

Given the many factors that can influence swallowing, it is not surprising that stroke-related dysphagia is highly variable in presentation between patients. Difficulties may be present in the pre-oral, oral, pharyngeal and/or oesophageal phases of swallowing. An example of pre-oral phase dysphagia may be 'impulsive' swallowing behaviours – poor monitoring of bolus size, rapid rate of ingestion, etc (Daniels, 2006). Oral phase dysphagia may be characterised by poor orolingual control of the bolus, including poor containment and preparation of the bolus prior to swallowing (Robbins & Levine, 1988). Pharyngeal phase dysphagia may present as any or all of the following: delayed initiation of swallowing (Robbins & Levine, 1988), reduced anterior movement of the hyoid bone during swallowing (Teasell, Foley, Fisher, & Finestone, 2002), reduced contact between the base of tongue and posterior pharyngeal wall during swallowing, poor pharyngeal motility (Horner & Massey, 1988). Finally, oesophageal phase dysphagia may be characterised by impaired opening of the upper oesophageal sphincter muscle during swallowing (Logemann & Kahrilas, 1990). Any or all of these symptoms may be present on a continuum ranging from mild impairment to profound impairment.

3.3. Consequences of Dysphagia

The consequences of dysphagia in the acute stages of stroke are potentially life threatening. For example, malnutrition and dehydration can hinder survival and recovery if left untreated (Dávalos et al., 1996). Dysphagia has been associated with poor patient outcomes, including poor nutritional state, poor recovery from stroke and increased level of dependence for care (Smithard et al., 1996). Guyomard et al. (2009)

reported odds ratios for increased length of hospitalisation and mortality of 3.9 and 12.5 among stroke patients if dysphagia was present.

Utilising alternative routes of nutrition can be a temporary solution to some of these problems. Examples include nasogastric tubes for nutrition, intravenous fluids for hydration and administering medications sublingually or rectally. These temporary measures are often replaced by oral intake as recovery progresses. However, the use of alternative routes of entry for administering medications is not always beneficial, as this can change drug metabolism and effectiveness (Dollery, Davies, & Conolly, 1971) and is associated with poor quality of life (Dennis, Lewis, & Warlow, 2005).

Another potentially life-threatening consequence of dysphagia is aspiration of food or fluid into the respiratory tract. Approximately 54 % of acute stroke patients experience aspiration of pharyngeal contents into the airway (Holas, DePippo, & Reding, 1994). Aspiration is a leading cause of pneumonia during acute hospitalisation and carries an odds ratio for death of 4.35 (Power et al., 2009). Importantly, even when oral feeding is completely replaced with nasogastric tube feeding, 31 - 44 % of patients still develop pneumonia (Dziewas, 2004; Mamun & Lim, 2005). Prevention of pneumonia is a key goal of acute stroke intervention.

A systematic review iby Martino et al. in 2005 reported the worldwide incidence of pneumonia following stroke. Internationally, the rate of pneumonia in the general stroke population was between 2 % to 16 %. In patients with dysphagia following stroke this increased to a range of 16 % to 19 %, with the exception of one study at 33 %. An earlier study that was not included in the review by Martino et al. reported a 44 % incidence of aspiration pneumonia, however a strict exclusion criterion of admission to hospital within 24 hours of stroke onset may have excluded patients with less severe strokes, resulting in selection bias (Dziewas, 2004).

Pneumonia continues to represent a significant healthcare issue, prompting the need to examine post-stroke dysphagia management practices.

Attempting to prevent the development – and recurrence – of pneumonia relies on an understanding of the aetiology. The aetiology of pneumonia is explored below with reference to specific risk factors.

3.4. Pneumonia

Pneumonia can be broadly divided into two categories: community-acquired and nosocomial (hospital-acquired). As the name suggests, community-acquired pneumonia (CAP) originates in the community. The infection is acquired via social contact. By contrast, nosocomial pneumonia can be defined as an infection that develops within 48 hours of admission to an institution such as a hospital or rest home. There are several different subtypes of nosocomial pneumonia, with distinctive aetiologies and underlying bacterial causes (Table 1). Aspiration pneumonia is of particular interest, given its association with dysphagia.

3.4.1. Aspiration pneumonia.

As its name suggests, aspiration pneumonia results from the introduction (aspiration) of food, fluid, or secretions that are colonised with pathogenic bacteria, at or below the level of the vocal folds. It is a common consequence of dysphagia among patients with neurologic injury. It should be noted that aspiration pneumonia differs from aspiration pneumonitis, which is a chemical injury to the lungs (Marik, 2001). The focus of this thesis will be on aspiration pneumonia.

3.4.2. Diagnosis of aspiration pneumonia.

A widely accepted definition of AP is an infective pneumonia that occurs in patients with a predisposition for aspiration (Lim et al., 2009; Mandell et al., 2007) following the inhalation of secretions colonised with pathogenic bacteria into the lower

Table 2

Comparison of Nosocomial Pneumonia Subtypes with Reference to Affected Populations and Dominant Pathogens

Nosocomial Pneumonia Subtype	Population affected	Dominant Pathogens	Reference
Ventilator-associated pneumonia	Intensive care unit patients requiring mechanical ventilation	*Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Staphylococcus aureus* *Escherichia coli* *Legionella pneumophila* *Streptococcus pneumoniae* *Enterobacter spp.*	American Thoracic Society, 2005; Bousbia et al., 2012; Esperatti et al., 2010; Talon et al., 1998
Non-ventilator intensive care unit pneumonia	Intensive care unit patients not requiring mechanical ventilation	*P. aeruginosa* *S. aureus*	Esperatti et al., 2010

Healthcare-associated pneumonia	Patients who have been hospitalized or who reside in a rest home; patients with underlying disease; patients who have received parenteral antimicrobial therapy, chemotherapy, or wound care	*S. pneumoniae* *L. pneumophilia* *P. aeruginosa* *Aspergillus spp.* *Acinetobacter spp.* *Enterobacteria spp.* *Klebsiella spp.*	American Thoracic Society, 2005; Kollef et al., 2005; Sopena & Sabrià, 2005
Aspiration pneumonia	Patients with dysphagia secondary to neurologic injury	*S. pneumoniae* *Streptococcus mitis* *Enterobacter clocae* *E. coli* *K. pneumoniae* *S. aureus*	Bousbia et al., 2012; El-Solh et al., 2003; Marik & Careau, 1999; Terpenning et al., 2001

respiratory tract (Marik, 2001). There is currently no gold standard test to diagnose AP. The British Society for Antimicrobial Chemotherapy (2008) and American Thoracic Society (2005) have provided guidelines for the diagnosis of hospital-acquired pneumonia (Table 2), although the latter guidelines do not take into account the quality of the evidence-base. Within the field of dysphagia, Mann et al. (1999) provide the most thorough and often-cited criteria for pneumonia, although there is difficulty extending these criteria to non-hospital populations where measures such as arterial blood gas are not routinely conducted. Other commonly cited criteria for diagnosing pneumonia in patients with dysphagia are listed in Table 2.

3.4.3. Aetiology of aspiration pneumonia.

In a landmark study in 1998, Langmore and colleagues described the multifactorial and complex aetiology of aspiration pneumonia. They analysed specific risk factors in 189 male patients recruited from acute wards, nursing homes and outpatient clinics over a four-year period. Patients from intensive care units were not recruited, and few patients were recruited from post-surgical units. Dysphagia was not an inclusionary or exclusionary criterion in this study.

The contributions of several specific risk factors in the development of aspiration pneumonia were evaluated. Participants with non-aspiration pneumonia were excluded from analysis but it is unclear how these participants were identified. Risk factors included dysphagia (as evident on videofluoroscopy and/or endoscopy), functional status, medical status and oral/dental status. Several variables related to oral bacteria, including number of decayed teeth and number of medications (leading to side effects such as reduced salivary flow and subsequent increased bacteria concentration), were included in the analysis.

Table 2.

Diagnostic Criteria for Hospital-acquired Pneumonia from International Guidelines and Selected Journals Relating to Patients with Dysphagia

Reference	Major criterion	Additional criteria
American Thoracic Society (2005)	New infiltrates on chest x-ray	Two of: purulent secretions, fever (>38 °C), leukopenia/leucocytosis.
The British Society for Antimicrobial Chemotherapy (Masterton et al., 2008)	New infiltrates on chest x-ray or purulent secretions	Fever (>38.3 °C), increased oxygen requirements, white cell count >10 or <4.
Langdon, Lee, & Binns (2009)		At least three of: fever (> 38 °C), abnormal respiratory examination (tachypnea > 22 breaths/minute, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal chest x-ray, arterial hypoxaemia (PO_2 < 70 mmHg), isolation of a relevant pathogen (positive result on Gram stain and culture).

Yeh et al. (2011)	Rales in breath sounds/dullness in chest percussion, or evidence of new infiltrate, consolidation, cavitation or pleural effusion on chest x-ray	At least one of: purulent sputum, positive sputum culture, positive blood culture
Bax, McFarlane, Green, & Miles (2014)		At least three of: fever (> 38 °C), abnormal respiratory examination (tachypnea > 22 breaths/minute, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal chest x-ray, arterial hypoxaemia (PO_2 < 70 mmHg), isolation of a relevant pathogen (positive result on Gram stain and culture).
Bravata et al. (2009)		Clinician diagnosis of pneumonia or fever, cough, shortness of breath, evidence of pneumonia on chest x-ray
Dziewas (2004)		At least three of: fever (> 38 °C), abnormal respiratory examination (tachypnea > 22 breaths/minute, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal

		chest x-ray, arterial hypoxaemia (PO_2 < 70 mmHg), isolation of a relevant pathogen (positive result on Gram stain and culture).
Gandolfi et al. (2014)		At least three of: fever (>38 °C), abnormal chest x-ray, abnormal respiratory examination, productive cough with purulent sputum, combined antibiotic therapy
Mamun & Lim (2005)	New infiltrate on chest x-ray	Chest symptoms (new/worsening cough, choking, shortness of breath, bronchospasm), and raised white blood cell count or fever
Gordon, Hewer, & Wade (1987)	Cough or fever	Evidence of new infiltrates on chest x-ray.
Masiero, Pierobon, Previato, & Gomiero (2008)		At least three of: fever (> 38 °C), abnormal respiratory examination (tachypnea > 22 breaths/minute, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal chest x-ray, arterial hypoxaemia (PO_2 < 70 mmHg), isolation of a relevant pathogen (positive result on Gram stain and culture).

Mann et al. (1999)	At least three of: fever (> 38 °C), abnormal respiratory examination (tachypnea > 22 breaths/minute, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal chest x-ray, arterial hypoxaemia (PO_2 < 70 mmHg), isolation of a relevant pathogen (positive result on Gram stain and culture).
Miles, Zeng, McLauchlan, & Huckabee (2013a)	At least three of: fever (> 38 °C), abnormal respiratory examination (tachypnea > 22 breaths/minute, tachycardia, bronchial breathing, inspiratory crackles), productive cough with purulent sputum, abnormal chest x-ray, arterial hypoxaemia (PO_2 < 70 mmHg), isolation of a relevant pathogen (positive result on Gram stain and culture).

The highest incidence of AP occurred in rest-home patients (44 %), followed by hospitalised patients (19 %) and patients living in their own home (9 %). Of those who developed AP, large proportions were post-stroke (27 %), had 'other' neurological disease (33 %) or had respiratory, heart or gastrointestinal disease (32 %). A diagnosis of both respiratory and gastrointestinal disease carried nearly a 50 % incidence of AP. Not surprisingly, patients with no underlying medical problems did not develop AP. Activity levels also played a role in the development of AP. Among participants who developed AP, 16 % were bed-bound and 43 % had reduced activity levels. These proportions were significantly higher than the corresponding values for patients who did not develop AP. Results contribute to an overall picture of general independence and physical health as likely protective factors against AP. The mechanism for this may be related to increased levels of oral pathogens in the dependent elderly (Russell, Boylan, Kaslick, Scannapieco, & Katz, 1999; Sumi, Miura, Michiwaki, Nagaosa, & Nagaya, 2007; Sumi, Miura, Sunakawa, Michiwaki, & Sakagami, 2002).

There was a significant difference between patients who developed AP and those who did not with regard to the presence of dysphagia. Of patients who developed AP, 81 % were diagnosed with dysphagia. Among participants with dysphagia, 58 % aspirated liquid, 27 % aspirated food and 50 % aspirated secretions. These proportions were significantly lower amongst patients who did not develop AP. The presence of dysphagia and aspiration were significantly related to AP development, primarily amongst acute care patients. Other aspects of dysphagia such as delayed initiation of pharyngeal swallowing, premature spillage and post-swallow pharyngeal residue were also significantly related to AP.

Despite these significant associations between presence of dysphagia and development of AP, dysphagia did not emerge as an independent predictor of AP in the multiple logistic regression model. The best independent predictors for aspiration pneumonia in orally-fed, dentate patients were dependence for feeding (OR = 11.8 [OR = odds ratio]) and multiple co-morbidities (OR = 7.3). When edentulous patients were also considered, dependence for feeding remained the most significant risk factor for AP (OR = 20.0), alongside multiple medications (OR = 1.2) and current smoking (OR = 4.1). When tube-fed patients were also included, significant risk factors were dependence for oral care (OR = 2.8) and tube-feeding (OR = 1.0).

Of particular interest was the finding that dysphagia alone was not sufficient to cause aspiration pneumonia. In a person who is medically stable, active and independent in activities of daily life (especially feeding) and who has a clean and healthy mouth, dysphagia and aspiration may not be problematic in terms of pneumonia risk (Langmore et al., 1998). This theory is supported by the fact that 45 % of healthy adults aspirate during sleep (Huxley, Viroslav, Gray, & Pierce, 1978) yet do not go on to develop aspiration pneumonia.

The heterogeneity of the participant cohort in the Langmore et al. (1998) study can be considered both a strength and a weakness. On one hand, the results can be considered to be broadly generalizable to several different patient populations, including those in acute care as well as the more stable, nursing home residents. On the other hand, the study cohort was not so heterogeneous that results can apply to all patient groups. By including few post-surgical patients, no female participants and no participants with past/present head and neck cancer, the opportunity to identify population-specific risk factors, if any existed, was lost. Although the inclusion of control participants adds a useful comparison, the large imbalance in size between the

'study' group (n = 160) and control group (n = 29) makes it difficult to draw meaningful comparisons.

Since the study by Langmore et al. (1998), several other research groups have attempted to identify independent risk-factors for AP that are specific to the acute stroke population. Among the findings, severity of stroke/stroke-related impairment, increased age, history of hypertension, low serum albumin, dysarthria or aphasia, diabetes mellitus, cognitive impairment, male gender and dysphagia have all emerged as independent risk factors for AP (Aslanyan, Weir, Diener, Kaste, & Lees, 2004; Dziedzic et al., 2006; Hinchey et al., 2005; Kammersgaard et al., 2001; Sellars et al., 2007). Studies to date, however, are limited by a lack of blinding during outcome measurement (Aslanyan et al., 2004; Dziedzic et al., 2006; Hassan et al., 2006), lack of heterogeneity among stroke sub-categories (i.e. infarction versus haemorrhage [Sellars et al., 2007]), lack of instrumental examination of swallowing (Sellars et al., 2007), lack of differentiation between community- and hospital-acquired pneumonia (Hinchey et al., 2005; Sellars et al., 2007) and a lack of robust methods for collecting pneumonia-related outcome measures (Sellars et al., 2007). Perhaps the greatest limitation of these studies is the restricted number of covariates taken into consideration (Dziedzic et al., 2006). Until a study that addresses these limitations is completed, it is difficult to say whether dysphagia is a true independent risk factor for AP in acute stroke patients. Regardless, the literature is in agreement that it is an important factor in the pathogenesis of AP.

Langmore et al. (1998) used broad criteria to define pneumonia: fever, elevated white blood cell count and evidence of a new change on chest radiograph. This may have contributed to the high, observed rate of pneumonia at 22 %. While diagnosis of pneumonia was made by a team of three physicians including a

pulmonologist, one could argue that the criteria used were broad and lacked specificity, as fever and increased white cells are symptomatic of many kinds of infection and chest infiltrates are not unique to pneumonia. Unfortunately, it is impossible to compare pneumonia rates between studies that have used alternative diagnostic criteria due to the wide variability in applied exclusion criteria and confounding patient variables.

Langmore et al.'s (1998) findings represented an important shift in the way clinicians approach dysphagia management in the acute and sub-acute phases of recovery. In the past, emphasis has been placed on dysphagia as the most important risk factor for aspiration pneumonia. By this line of thinking, keeping patients nil by mouth (NBM) and utilising alternative routes of nutrition and hydration or, alternatively, cautious assisted feeding, would be the best way to prevent AP. However, the evidence presented by Langmore et al. challenged this school of thought. In fact, their results suggested quite the opposite – that tube-feeding and dependence for feeding were two of the most significant risk factors for AP and presence of dysphagia was not. Langmore et al. proposed a model for the development of pneumonia, suggesting that a feature from each of the following must be present in order for pneumonia to develop: a) the presence of pathogenic bacteria in the upper aerodigestive tract, b) aspiration of pathogenic bacteria into the lungs and c) an inability to clear the aspirate, leading to bacterial colonisation and subsequent lower respiratory tract infection. Controlling these three factors may be one way of preventing aspiration pneumonia from developing in high-risk populations. Yet despite these findings being published almost 20 years ago, the role of bacteria and airway protective mechanisms are two areas of stroke research that remain relatively under-explored. Understanding these factors may have direct implications for how

patients with acute stroke are assessed for risk of AP as well as the management of patients with stroke-related dysphagia in the acute setting.

3.5. Summary

Stroke is a highly prevalent condition and a leading cause of adult long-term disability. Dysphagia is a common consequence of acute stroke (Daniels & Foundas, 1999; Gottlieb, Kipnis, Sister, Vardi, & Brill, 1996; Kidd, Lawson, Nesbitt, & MacMahon, 1993; Langdon, Lee, & Binns, 2007; Mann, Hankey, & Cameron, 1999; Marik & Kaplan, 2003; Martino et al., 2005; Meng, Wang, & Lien, 2000) that increases the risk of pneumonia and death (Power et al., 2009; Sellars et al., 2007). For many patients, swallowing function eventually resolves. However, persisting dysphagia has been documented in stroke patients after six months (Mann et al., 1999; Smithard et al., 1997). Because stroke-related dysphagia is highly variable in presentation between patients, a thorough understanding of the risk factors for pneumonia is needed to adequately assess and manage this patient population. Dysphagia, when accompanied by increased oral bacteria, aspiration and poor pulmonary clearance, may substantially increase the risk of pneumonia in patients with acute stroke. Particular attention should be given to understanding the relationships between these risk factors and how they could be modified to decrease the risk of aspiration pneumonia.

Chapter 4. Assessments and Interventions to Reduce the Incidence of Aspiration Pneumonia in the Acute Stroke Population

4.1. Silent Aspiration: A Clinical Conundrum

Langmore et al. (1998) identified aspiration of pathogenic bacteria into the lungs as a precursor to aspiration pneumonia in patients with dysphagia. Videofluoroscopy (VF) is considered to be the gold standard in dysphagia assessment and identification of aspiration. However, due to costs associated with equipment, training and skilled staff as well as the radiation exposure involved in VF, it is not appropriate to screen every patient using this tool. For this reason, alternative screening assessments have been developed that can be performed at bedside with little disruption to the patient. Many of these assessments use coughing as a clinical indicator, where the presence of cough suggests aspiration. For example, the water swallow test (DePippo, Holas, & Reding, 1992; Gordon, Hewer, & Wade, 1987; Smithard et al., 1998, etc) defines aspiration as the presence of coughing during or up to one minute after swallowing certain amounts of water. The presence of wet dysphonia may also be used as a clinically positive indicator of aspiration during this test. Unfortunately, in using such tools, identification of aspiration can often be missed in patients who present with silent aspiration, defined as aspiration in the absence of a reflexive cough.

An historic study by Splaingard and colleagues (Splaingard, Hutchins, Sulton, & Chaudhuri, 1988) reported that 42 % of patients who aspirated did so silently. Of utmost concern is that 70 % of patients with severe aspiration (as observed on instrumental assessment) were not identified as “at risk” on clinical assessment. In

other words, patients who were most at risk were also the least likely to be identified on clinical assessment (Splaingard et al.). Subsequent large-scale studies have supported this finding with very similar results (Garon, Ormiston, & Sierzant, 2009; Smith, Logemann, Colangelo, Rademaker, & Pauloski, 1999).

Clinicians are faced with a significant challenge: how to identify silent aspirators at bedside? In an attempt to meet this challenge, several research groups have investigated the use of cough reflex testing (CRT [Addington, Stephens, & Gilliland, 1999; Leow, Beckert, Anderson, & Huckabee, 2012; Miles et al., 2013a; Miles et al., 2013b; Moore & Huckabee, 2013; Wakasugi et al., 2008; Watando et al., 2004]).

4.2. The Reflexive Cough Response

Intact airway protection mechanisms are essential to prevent the development of aspiration pneumonia. The swallowing and cough reflexes, when intact, serve to protect the airway from aspiration of potential pathogens, and the attenuation of these reflexes is a main contributor in the development of aspiration pneumonia (Yamaya, Yanai, Ohrui, Arai, & Sasaki, 2001). For example, it has been reported that patients with stroke who show both a latency of reflexive swallowing of greater than five seconds and decreased cough reflex sensitivity have a higher incidence of pneumonia (Nakajoh et al., 2000).

Cough reflex testing has been utilised in the field of respiratory medicine since the 1950s (Bickerman, Barach, & Drimmer, 1954). More recently, the application of CRT to clinical dysphagia assessment and management has received attention, as research has revealed significant relationships between pneumonia and reduced voluntary cough strength (Pitts, Bolser, Rosenbek, Troche, & Sapienza, 2008; Smith Hammond et al., 2009), reduced laryngo-pharyngeal sensation and reflexive airway

closure (Aviv et al., 1997) and reduced reflexive cough sensitivity (Addington, Stephens, & Gilliland, 1999; Miles et al., 2013a; Nakajoh et al., 2000; Niimi et al., 2003), in patients both with and without impaired neurology.

The role of CRT in clinical practice is to identify patients *at risk* of silent aspiration. It is important to note that a patient who presents with an abnormal response to CRT (e.g. "weak" or "absent" cough) cannot be labelled a "silent aspirator" as CRT does not involve the ingestion of any food/fluid to be silently aspirated and silent aspiration can only be identified through observation of internal structures i.e. via videofluoroscopy or nasoendoscopy. Rather, in the event of aspiration, an abnormal response to CRT may increase a patient's risk of silent aspiration.

The test itself involves inhalation of a tussive agent delivered via a nebuliser. In clinical practise, the outcome is measured by a subjective judgement of cough presence +/- cough strength. The European Respiratory Society Council (ERS) have published a recommended protocol (Morice et al., 2007). However, several methods of CRT have been described in the literature; to date there is no universally accepted method for research or clinical purposes.

4.2.1. Cough reflex testing in dysphagia management.

The application of CRT in clinical dysphagia management is fairly recent. As previously mentioned, CRT is an appealing tool as it is inexpensive, quick and non-invasive compared to traditional assessment tools such as videofluoroscopy and videoendoscopy (VE). For use in screening, however, CRT must have acceptable sensitivity and specificity for detecting silent aspiration.

Wakasugi et al. (2008) investigated the usefulness of CRT as a screening tool for silent aspiration at bedside by validating results from CRT against results from an

instrumental assessment, either videofluoroscopic swallowing study (VFSS) or videoendoscopic evaluation of swallowing (VEES). Participants (n = 204) ranged in age from 18 to 100 years, with a mean age of 70 years. Participants were a heterogeneous group that included both inpatients and outpatients with suspected dysphagia. Each participant underwent CRT and instrumental assessment, performed on the same day or within one day of each other. The CRT involved patients inhaling nebulised citric acid (1.0 w/v %) orally for one minute while their cough response was monitored. Five or more coughs was considered a 'pass' (normal) and less than four coughs was considered a 'fail'. The result of precisely four coughs was not directly stated.

For approximately half of the participants, CRT results were combined with findings from another bedside diagnostic test: the modified water swallowing test (MWST [Tohara, Saitoh, Mays, Kuhlemeier, & Palmer, 2003]). The MWST consisted of a 3 mL aliquot of water placed on the floor of mouth. Participants were instructed to swallow the bolus followed by two saliva swallows. This procedure was repeated three times. The test was scored depending on the participants' swallowing response, ranging from no response, to post-swallow dyspnea, to coughing and/or wet vocal quality. Participants suspected of severe aspiration did not undergo the MWST.

The CRT alone had a positive predictive value (PPV) of 0.74 and a negative predictive value (NPV) of 0.95. Sensitivity and specificity of the CRT for detecting silent aspiration was .87 and .89, respectively. Combining the CRT with the MWST improved the accuracy of dysphagia diagnosis. Using this protocol, 89 % of the predicted normal group were also found to be normal on instrumental examination, 73 % of the predicted aspiration with cough group actually aspirated and coughed, and 88 % of the predicted silent aspiration group were actually silent aspirators. The

protocol did, however, fail to identify one silent aspirator, and five patients who only silently aspirated trace amounts of fluid. The authors interpreted these results as evidence for combining the CRT and MWST and incorporating these into dysphagia bedside screening protocols.

A difficulty that the authors of this study encountered was how to analyse results from participants who coughed on large amounts of aspirate but did not react to trace amounts during VFSS. Initially, these participants were analysed separately from the silent aspiration group and the PPV and NPV of the CRT was very high. However, even when this group was re-analysed as silent aspirators, the PPV and NPV was 0.77 and 0.84, respectively. Regardless of how trace aspiration is interpreted, these results suggest that the CRT can predict silent aspiration with high levels of accuracy.

Patients in the Wakasugi et al. (2008) study were assigned to receive either VFSS or VEES to evaluate their swallowing. However, specific details regarding how this decision was made as well as comparisons between the two groups in terms in terms of dysphagia severity, aspiration, patient characteristics and CRT/MWST results were not reported. This may be problematic for clinicians attempting to generalise these findings to their own practice. VEES may result in a higher incidence of perceived aspiration compared to VFSS (Butler, Stuart, & Kemp, 2009). Results from data obtained using VFSS and VEES should not be pooled without first controlling for this. Another limitation of the Wakasugi et al. study was the lack of justification for the citric acid dosage. The clinical validity of the dosage used by Wakasugi et al. is unknown.

Miles et al. (2013b) addressed this in a validity study that included doses of citric acid that were based on normative data (Monroe et al., 2014). Cough reflex

thresholds from 181 hospitalised patients referred for instrumental swallowing assessment were measured. Patients were allocated to receive either a VFSS (n = 80) or a VEES (n = 101). The prevalence of silent aspiration was 21 % for patients who received VFSS and 57 % for patients who received VEES. Sensitivity and specificity differed between the different types of instrumental assessment and at different doses of citric acid. Among patients who aspirated, for VFSS studies, the optimal dose of citric acid that identified a silent aspirator was 0.6 mol/L (71 % sensitivity and specificity). Among patients who aspirated, for VEES, the optimal dose of citric acid that identified a silent aspirator was 0.4 mol/L (69 % sensitivity, 71 % specificity). For both tests, sensitivity/specificity improved when trace aspirators were removed from the analysis and decreased when the non-aspirators were included in the analysis. The fact that the optimal citric acid dose differed between VFSS/VEES studies likely reflects that patients in the VEES group were older and more acutely unwell than the VFSS group and is a limitation of the study.

Sensitivity/specificity values of the CRT reported by Wakasugi et al. (2008) and Miles et al. (2013b) compare favourably to other reported single measures of aspiration risk, suggesting that it is appropriate for use in clinical populations. As both studies reported findings from heterogeneous patient cohorts it remains unknown whether there is an optimum dose of citric acid that differs between patient populations.

Sato et al. (2012) completed a validation study of CRT methods using a hand-held mesh nebuliser and citric acid in 141 adults with suspected dysphagia. Participants were a heterogeneous cohort, with the majority having cerebrovascular disease. The primary outcome measure was the time taken between presentation of citric acid (1 mol/L) and the participants' first cough. Citric acid was presented

through a mouthpiece for a maximum of one minute. Participants also underwent a VEES within two days of the CRT and outcomes relating to aspiration were recorded. Aspiration was observed in 53 participants with silent aspiration in 37. Presenting citric acid for 60 seconds provided the best diagnostic accuracy for detecting silent aspiration with a sensitivity of 81 % and specificity of 65 %. Among the participants who aspirated, 30 seconds provided the best diagnostic accuracy from distinguishing silent aspirators from overt aspirators. While these results provided evidence for CRT methods, the study could have been strengthened by selecting a citric acid dose that was based on normative or clinical data. The use of a mouthpiece may also be problematic in neurologically-impaired populations where concomitant lip weakness/difficulty following instructions may be present.

Imoto, Kojima, Osawa, Sunaga and Fujieda (2011) attempted to characterise a cough reflex threshold level that would accurately inform safety for oral intake in a group of 21 patients with neurogenic dysphagia and twelve healthy control participants. Although a detailed description of their CRT technique was not provided, threshold testing using fifteen-second trials of capsaicin (0.5 – 250 μM) was completed. The main outcome measures were cough reflex threshold (as measured using the C_5 criterion) and dysphagia severity (as measured by diet recommendation following VEES or VFSS). Dysphagia severity was classified as normal (i.e. no dysphagia), mild (full oral intake), moderate (oral intake with supplemental nutrition/hydration) or severe (no oral intake). Dysphagia severity was shown to have a moderately strong correlation to cough threshold level. Participants with normal swallowing had a cough threshold of 0.98 – 7.8 μM capsaicin, mild dysphagia correlated to a cough threshold of 1.95 – 15.6 μM, moderate dysphagia correlated to a cough threshold of 15.6 – 62.5 μM and severe dysphagia correlated to a cough

threshold of 31.2 – 250 μM. Despite the differences between groups being statistically significant, there is limited clinical significance of these findings due to considerable overlap in the range of capsaicin thresholds. Measuring dysphagia severity by diet recommendation levels is fraught with bias that comes with clinician experience and individual practice patterns, familiarity with the patient and within-patients factors such as dentition and food/texture preferences. CRT assesses laryngeal sensory integrity only and as such, a more appropriate criterion reference would be to measure presence/response to aspiration as viewed under videoendoscopy or videfluoroscopy.

More recently, Wakasugi et al. (2014) investigated the usefulness of a small, hand-held nebuliser in a similar heterogeneous cohort of inpatients and outpatients with suspected dysphagia. The study design and methodology were essentially the same as the 2008 study (Wakasugi et al., 2008) with the exception that the MWST was not used for dysphagia screening. Similar high sensitivity (0.86) and specificity (0.71) of the CRT were calculated for identifying silent aspiration. Positive predictive value was lower at 0.78 and negative predictive value was 0.93. Results confirmed previous findings that citric acid CRT can be used as a screening for silent aspiration risk in heterogeneous populations with acceptable sensitivity and specificity (Miles et al., 2013b; Wakasugi et al., 2008). However, the later study by Wakasugi et al. (2014) had the same limitations as earlier work, namely a lack of detail and comparative data between participants who received VFSS versus VEES and the pooling of data taken using these different assessments.

These limitations were addressed in a recent validation study by Guillén-Solà et al. (2015). First-event, subacute patients with stroke (N = 134) and suspected dysphagia underwent both CRT and VFSS. The CRT method involved presenting a single dose of citric acid (1 mol/L) for one minute and counting the number of coughs

produced by the patient. A result of four or fewer coughs constituted a 'fail' result. The nebuliser was an ultrasonic nebuliser with a flow rate of 3 mL/min. Specific details regarding the method of presentation (e.g. face mask, mouthpiece) were not reported. Patients underwent VFSS immediately following CRT. VFSS outcomes were measured using the Penetration-Aspiration Scale (Rosenbek, Robbins, Roecker, Coyle, & Wood, 1996) by a researcher who was blinded to the patients' CRT result. Silent aspiration was identified on VFSS in 26 participants (19 %). Of this group, only five participants had failed the CRT. Out of the total number of patients who failed the CRT (n = 36, 27 %), five participants (14 %) were identified as silent aspirators on VFSS, 12 participants (33 %) were identified as normal, 14 participants (39 %) were penetrators and five participants (14 %) were overt aspirators. Sensitivity of the CRT for identifying silent aspiration was 0.19, specificity was 0.74, positive predictive value was 0.5 and negative predictive value was 0.4. The authors concluded that the single-dose method of CRT using citric acid at a concentration of 1 mol/L is not an appropriate screen for silent aspiration in subacute stroke patients.

There are several limitations of this study. Inclusion criteria for the study was suspected dysphagia based on the results of a volume-viscosity swallowing test (Clavé et al., 2008). This test relies on several subjective judgements (i.e. observed coughing, change in vocal quality) as well as objective measures (i.e. oxygen desaturation) to identify aspiration. However, given that there is little convincing evidence that a change in vocal quality is a reliable indicator of penetration/aspiration and that oxygen desaturation is not considered to be a valid marker of aspiration (Steele & Cichero, 2014), it is concerning that this was the main inclusion criteria for the study. It is likely that a selection bias that limited the number of patients with silent aspiration was a factor in this study, although the reported prevalence of 35 % is

similar to other published reports of 38-39 % in subacute dysphagic patients (Holas et al., 1994; Horner & Massey, 1988). Decreased cognition was an exclusion criterion for this study, which may also contribute to a selection bias and decrease the generalizability of results. Another explanation for the low sensitivity observed by Guillén-Solà et al. (2015) could be related to trace aspirators. Previous researchers have noted that people who present trace silent aspiration during VFSS are more likely to pass CRT compared to people who silently aspirate larger amounts (Wakasugi et al., 2008) and higher sensitivity of CRT is achieved when trace aspirators are removed from data analysis (Miles et al., 2013b; Wakasugi et al., 2008). It is not clear from the data reported by Guillén-Solà et al. (2015) whether sensitivity of the CRT may have been affected by a high proportion of trace silent aspiration.

Wakasugi et al. (2008), Miles et al. (2013b) and Wakasugi et al. (2014) demonstrated that CRT can identify patients who silently aspirate with reasonable sensitivity and specificity. In terms of cost, patient comfort, and time taken to administer, CRT presents as a clinically useful alternative to many other single measures in identifying those patients most likely to benefit from further instrumental swallowing evaluation. The next logical question is: how does using CRT translate to reduced end-point pneumonia rates in patients with dysphagia?

Addington, Stephens, and Gilliland (1999) compared 602 consecutive acute stroke patients from two different hospitals to examine whether the use of CRT would differentiate patients who developed pneumonia from those who did not. The CRT protocol involved the use of nebulised tartaric acid (20 % solution *l*-tartaric acid dissolved in 2 mL saline) that participants inhaled as a microaerosol through a mouthpiece at the bedside. Data were not included when there was leakage around the mouthpiece. The CRT concluded when either a) a cough response was elicited, or b)

there was no response after three trials. An algorithm was provided to Speech-Language Therapists (SLTs) to guide their interpretation of CRT results. Specifically, where participants demonstrated a strong cough and had adequate cognition (subjective judgement relating to the participant's capacity to follow compensatory strategies), the SLT was advised to complete the bedside swallowing exam, including presentation of oral trials. Where participants did not have intact cognition, SLTs were advised to proceed with the bedside exam, but consider alternative feeding (i.e. nasogastric tube [NGT], percutaneous endoscopic gastrostomy [PEG]). Where participants presented with an absent or weak cough, the SLT was advised to recommend a restricted diet, NBM, or alternative feeding. The procedure for standard clinical swallowing evaluation was described, including evaluation of cranial nerves, cognition, volitional cough, a two-part water swallowing test, post-swallow vocal quality, and trials of foods and fluids of varying texture/consistency.

Participants were followed for the duration of their acute stay (average 9-10 days) and outcomes related to pneumonia recorded. Within the CRT group, 1 % developed pneumonia versus 13 % of the no-CRT group ($p < .001$). The authors concluded that CRT was effective in identifying pneumonia risk in patients with acute stroke and could be used to prevent the development of pneumonia by informing decisions around feeding.

The results of this study are significant both clinically and statistically (high statistical power was reported). However, there were also several significant limitations in methodology. The major limitation was the absence of a true control group. Confounding variables such as differing oral care practices, patient meals, hygiene practices, medical management, etc, combine with significant effect to decrease the validity of results. Furthermore, the authors mention that an inadequate

lip seal around the mouthpiece during CRT was not considered to be an "effective inhalation", however it is unclear how many patients could not could not achieve an adequate lip seal and were excluded from data analysis. In a clinical population, it is expected that a substantial proportion of patients may present with a poor lip seal due to unilateral hemiplegia/hemiparesis, dystonia of the orbicularis oris muscle, poor attention to the task, etc. If high numbers of patients were excluded from this study the results lose generalizability and significance. This may also account for the low reported pneumonia rates. Finally, the authors did not report their reasons for selecting the dosage of tartaric acid that was used in the study and do not cite normative data, leaving the reader unclear about what constitutes a clinically abnormal response and subsequently, whether patients' responses to CRT were normal/abnormal. However, the early work by Addington et al. (1999) has paved the way for subsequent studies, which has ultimately improved the body of knowledge and quality of evidence in this area.

One such study was completed by Miles et al. (2013a), who completed a Phase III randomised controlled trial that sought to further evaluate the use of CRT in standard clinical practice. Patients with acute stroke from four metropolitan hospitals ($N = 311$) were randomised to either a) a control group who received usual care in terms of swallowing assessment and management according to local clinical protocols, or b) an experimental group who underwent CRT in addition to usual care, with CRT results informing subsequent swallowing management. Results revealed that patients who underwent CRT had a higher frequency and speed of referral for instrumental assessment (VFSS or VEES), compared to patients in the control group. However, in contrast to Addington et al. (1999), the use of CRT did not significantly reduce pneumonia or mortality rates.

The authors suggested that this result may have been in part attributable to a heavy reliance on clinician judgement and interpretation of CRT findings in the absence of a defined management protocol, such as was seen in the Addington et al. (1999) study. Qualitative analysis of clinician comments as well as anecdotal evidence suggested that routine clinical practice was often favoured over decisions that incorporated the novel CRT results, which may well have resulted in unfavourable patient outcomes. Indeed, this could be seen in the rate of referral for instrumental assessment amongst patients who 'failed' CRT (i.e. had an absent reflexive cough) – merely 46 %. In other words, 64 % of patients with absent reflexive cough were initially managed based on clinical impression alone.

The findings from Miles et al. (2013a) suggest that clinicians' ability to identify aspiration risk does not necessarily result in the prevention of aspiration pneumonia in their patients. In order for this to occur, assessment findings must be reflected in management decisions.

Several considerations need to be taken into account when applying CRT to clinical or research applications, including the different types of equipment, tussive agents, method of administration, instructions given to the subject, within-subject diurnal variation in cough response, placebo effects, the effects of tachyphylaxis and the effects of medications on reflexive cough. These are explored below.

4.2.2. Equipment.

Nebuliser flow rate has been found to impact cough response to citric acid by altering the deposition of aerolised particles (Barros, Zammattio, & Rees, 1990). For this reason, the ERS recommend the same nebuliser (or nebulisers with identical output) be used for all research involving CRT, preferably a compressed air-driven nebuliser

controlled by a dosimeter and inspiratory flow regulator valve that limits output to 0.5 L/second (Morice et al., 2007).

4.2.3. Administration of CRT.

Two main methods of CRT administration are described in the literature: vital-capacity breath inhalation (e.g. Addington, Stephens, & Goulding, 1999) and tidal-breath inhalation (e.g. Miles et al., 2013a). The vital-capacity breath inhalation method requires the subject to exhale and then take a deep inspiration through a mouthpiece that delivers the tussive agent. The subject's nose is pinched or blocked during the test and cough response is recorded. Flow rate is controlled via a dosimeter, which eliminates variation in intra-subject inspiratory effort. In contrast, the tidal-breathing method uses a facemask that covers the nose and mouth to deliver the tussive agent for a specific period of time at a specific flow rate. The subject's nose may or may not be blocked, and cough response is recorded. Both methods have advantages and disadvantages. As noted by Miles et al. (2013a), the tidal breathing method may allow more opportunity for cortical modulation of the cough reflex compared to the vital-capacity method which may be more likely to elicit a true reflexive cough. On the other hand, the tidal breathing method does not require compliance to a specific set of instructions (Miles et al., 2013a) and as such may be more appropriate for use in patient populations where language, cognition or oromotor function are impaired, such as in acute neurological settings. Fifteen-second presentations of the tussive agent is considered sufficient when the outcome of interest is number of coughs (Dicpinigaitis, 2007).

Aerosol deposition in the upper and lower respiratory tract has also been shown to vary depending on whether a person breathes through their nose, mouth, or through a mouth-tube (Wolfsdorf, Swift, & Avery, 1969). Nasal breathing and oral

breathing results in a greater proportion of particles deposited in the upper respiratory tract compared to breathing through a mouth-tube. In contrast, breathing through a mouth-tube deposits more particles in the lower respiratory tract compared to nasal or normal mouth breathing. As noted by Miles et al. (2013a), for application in dysphagia assessment, CRT incorporating nasal or mouth breathing may be preferable as the goal is to test integrity of laryngeal airway protection mechanisms rather than lower respiratory tract function.

4.2.4. Tussive agents.

The most commonly used tussive agents include tartaric acid (Addington, Stephens, & Gilliland, 1999), capsaicin (Dicpinigaitis, 2003a; Sams, Truncale, & Brooks, 2005), citric acid (Leow et al., 2012; Miles et al., 2013a; Miles et al., 2013b; Monroe, Huckabee, & Robb, 2010; Smith, Owen, Earis, & Woodcock, 2006; Wakasugi et al., 2008) and distilled water (Fontana, Lavorini, & Pistolesi, 2002). Of these, citric acid may be preferable for use in dysphagia assessment, as it is known to stimulate both the chemo- and mechano-receptors of the larynx (Morice et al., 2007) in a manner that is reproducible across time (Wright, Jackson, Thompson, & Morice, 2007), less susceptible to the effects of tachyphylaxis (Morice et al.) and has no reported adverse reactions (Bickerman et al., 1954).

To date, the only normative data available on response to citric acid cough reflex testing are from two observational studies reported in a single publication (Monroe, Manco, Bennett, & Huckabee, 2014). These data represent 160 healthy individuals with equal proportions of males, females, young (under 60 years) people and old (over 60 years) people. Cough reflex was tested in individuals by presenting nebulised citric acid via a face mask for up to 15 seconds. Both the natural cough reflex and suppressed cough reflex (i.e. participants were instructed to try to suppress

the cough) were measured, with two consecutive coughs on two out of three trials constituting a positive response. Data from these studies suggest that 5 % of healthy individuals may not produce a reliable cough response to citric acid when presented at concentrations of up to 1.2 mol/L and 22 % may be able to suppress the cough reflex beyond 1.2 mol/L. Among those in which a reflexive cough was triggered, 90 % of participants demonstrated a natural cough by 0.8 mol/L with 96 % of participants unable to suppress their cough reflex at 1.2 mol/L. The mean threshold for the natural cough reflex was 0.4 mol/L in both old and young participants. Female participants showed both significantly lower natural cough thresholds and suppressed cough thresholds compared to males.

4.2.5. Outcome measurement in CRT

Several methods of recording cough reflex outcome measures have been reported in the literature. In laboratory-based studies, objective measures such as recording cough airflow (e.g. Troche, Brandimore, Okun, Davenport, & Hegland, 2014), cough inspiratory volume and total expired volume (e.g. Hegland, Troche, & Davenport, 2013), compression phase duration and cough volume acceleration (e.g. Wheeler Hegland, Troche, Brandimore, Davenport, & Okun, 2014) have been described. Most clinical studies, however, simply rely on counting the number of coughs (Addington, Stephens, & Gilliland, 1999; Guillén-Solà et al., 2015; Miles et al., 2013a; Miles et al., 2013b; Wakasugi et al., 2008, 2014). The ERS Task Force recommend using either the C_2 or C_5 method (i.e. two or five consecutive coughs following presentation of the tussive [Morice et al., 2007]). As the response to the tussive agent is usually immediate, a fifteen-second measurement period is sufficient (Dicpinigaitis, 2007). As discussed above, this method has been shown to satisfactorily identify patients at risk of silent aspiration.

4.2.6. Safety of CRT equipment.

Typical nebulisers used in CRT generate small particles of 1 – 3 μm diameter that, when inhaled, can reach the terminal bronchioles and alveoli of the lungs. Since the 1960s, inhalation therapy equipment has been linked to cases of nosocomial pneumonia (Reinarz, Pierce, Mays, & Sanford, 1965). Pathogenic bacteria colonise the equipment and are subsequently inhaled into the lungs, leading to infection. There are important distinctions between the use of nebulisers for inhalation therapy as opposed to CRT. First, when CRT is applied per the protocol described by Miles et al. (2013a), the total time of inhalation is around 45 seconds, with frequent pauses throughout. This is less than the time it takes for bacteria to reproduce. By comparison, nebulisation therapy typically lasts several hours. Second, given that the equipment used in CRT is single-use only, the risk of contamination is likely to be lower than that used of inhalation therapy equipment, which is cleaned and re-used (Ombler & Huckabee, 2015). Using the same CRT equipment described in the literature (e.g. Miles et al., 2013a), Ombler and Huckabee (2015) reported no significant bacterial growth in the mask, reservoir cup or tubing when comparing pre- and post-nebulisation with citric acid. However, it should be noted that these results reflect a laboratory environment and a single, careful administrator of CRT, as opposed to a hospital setting with intra-individual variation in CRT practises. Falconer et al. (2014) investigated the stability of citric acid solution (0.8 M) stored at 4 °C and the sterility of two-week old citric acid from a hospital ward as well as citric acid that was inoculated with *C. albicans, E. coli* and *S. aureus*. Stability was maintained over 28 days. In the innoculated citric acid, *E. coli* and *S. aureus* were killed but *C. albicans* remained viable. There was no evidence of contamination of the ward-based citric acid. The authors recommend the use of aseptically prepared citric

acid in single-use aliquots to reduce the risk of accidental contamination. However, in the real-world condition of ward-based citric acid, no contamination was detected, suggesting that careful repeated opening of citric acid may be acceptable. It should be noted that only three organisms were tested for in only one concentration of citric acid. Further research is needed to clarify best practice around the storage and use of citric acid for CRT.

4.2.7. The impact of diurnal variation in CRT.

Another consideration in the administration of CRT is within-subject diurnal variation in cough reflex sensitivity. This was first described in the mid 1980s (Pounsford & Saunders, 1985). Cough sensitivity was measured in ten healthy participants (age range = 19 – 40 years) by presenting nebulised citric acid at varying concentrations (i.e. cough reflex threshold testing; CRTT) in the morning (between 9am – 12pm) and the afternoon (between 2pm – 5pm). The vital-capacity method was used. The lowest concentration of citric acid that elicited a reflexive cough was considered to be that participant's cough threshold. Testing was carried out over four days (two morning sessions and two afternoon sessions) and an average of the morning values and afternoon values was used in analysis. Two out of ten participants had no response to citric acid; their data were excluded from analysis. The remaining participants had significantly higher cough reflex thresholds in the afternoon, compared to the morning. An analysis of variance revealed no significant change in the mean cough reflex threshold over the four days. The authors concluded that diurnal variation in cough response to citric acid represents a significant potential confounding variable, which should be controlled for by testing at the same time of day. It remains unknown whether the same diurnal variation in cough reflex sensitivity is present or observable in dysphagic patient populations (e.g. acute stroke), whether such changes are

clinically significant or whether the effect is present when different CRT methods are employed. In research applications, controlling time of day may be more feasible. Indeed, when steps are taken to control time of day, cough reflex thresholds have been shown to remain constant within participants across time (Rees & Clark, 1983).

4.2.8. Placebo effects in CRT.

There is evidence for a placebo effect in evoked cough reflex testing. Rostrami-Hodjegan, Abdul-Manap, Wright, Tucker and Morice (2001) gave healthy subjects a placebo antitussive syrup followed by repeated presentations of 1 M citric acid via a face mask. Participants demonstrated an immediate suppression of the number of coughs, compared to baseline measures. This increase in cough suppression continued in a non-linear fashion to peak at 4 hours post-placebo administration, with females adapting their cough response significantly quicker than males ($p < .05$). Similar findings have been reported using capsaicin-induced cough testing (Hutchings & Eccles, 1994; Leech, Mazzone, & Farrell, 2012).

Findings such as those reported by Rostami-Hodjegan et al. (2001), Hutchings and Eccles (1994), and Leech et al. (2012) suggest that the cortex may play a significant role in the modulation of cough. Mazzone, Cole, Ando, Egan, and Farrell (2011) tested this theory using functional brain imaging in healthy participants who performed different tasks relating to cough: 1) inhalation of nebulised saline (control condition), 2) voluntary cough, 3) evoked cough in response to inhaled capsaicin, 4) suppressed cough in response to inhaled capsaicin. Results showed that, despite sharing many areas of cortical activation, voluntary cough, evoked cough and suppressed cough were each associated with unique areas of the cortex. For example, evoked cough was associated with activation of the posterior insula, posterior cingulate cortex, primary motor and somatosensory cortices, premotor cortex, and

medulla that was distinct from voluntary or suppressed cough, while activation of the lower brainstem was conspicuously absent in voluntary cough. Suppressed cough was associated with unique activation in the pre-supplementary motor area and caudate. The authors concluded that capsaicin-evoked cough is not purely a brainstem-mediated response to airway irritation, rather it involves active cortical involvement (an 'urge to cough') and is able to be regulated by higher level inhibitory functions. Studies of cortical activation such as this are, however, constrained by the fact that some of the observed cortical activation may relate to cognitive-language processing associated with the task.

With evidence such as Mazzone et al.'s (2011) in favour of complex cortical interactions during both reflexive and voluntary cough tasks, it has been proposed that a suppressed cough condition may give a more accurate picture of laryngeal sensitivity, as cortical inhibition can no longer override the brainstem response to cough (Hegland, Bolser, & Davenport, 2012; Monroe et al., 2010). This has implications for the instructions that are given to subjects undergoing cough reflex testing. Studies of evoked cough in both healthy subjects (Hutchings & Eccles, 1994; Leow et al., 2012) and subjects with Parkinson's Disease (Leow et al.) have demonstrated that subjects are able to inhibit or produce cough to a very large extent depending on the instruction they are given (i.e. "cough if you need to" vs. "try not to cough"). The ERS guidelines on the assessment of cough (2007) caution researchers to expect a significant placebo effect in research involving cough reflex testing and to design research studies accordingly. To rule out voluntary suppression of the cough and to increase participant blindness during threshold testing, the use of placebo presentations is recommended (Dicpinigaitis, 2007; Morice, 1996; Morice et al.,

2007). Instructing participants to suppress the cough may also be useful when assessing integrity of the reflexive airway protection mechanisms.

4.2.9. Effects of tachyphylaxis in CRT.

Short-term tachyphylaxis, or blunting of the reflexive cough response, has been documented where multiple doses of citric acid or capsaicin have been presented over short (e.g. one minute) periods of time (Morice, Higgins, & Yeo, 1992). This has implications for randomised, repeated presentation study designs, as it is likely that decreased sensitivity to lower citric acid concentrations would occur as a result of short-term tachyphylaxis (Morice, 1996). In order to avoid this, presenting citric acid in increasing increments with 30-second intervals is recommended, with placebo saline interspersed throughout (Morice et al., 2007; Morice, Kastelik, & Thompson, 2001). In addition, to avoid the significant adaptation effect that occurs between presentation one and presentation two (Morice et al., 1992), it is recommended for research that all participants are familiar with cough testing before measures are recorded, e.g. present a placebo saline dose prior to citric acid presentations.

4.2.10. Reproducibility in CRT.

Because the number of coughs that a person produces in response to evoked cough reflex testing varies between trials, it is recommended that a number of trials are performed in order to obtain a more accurate measure of cough frequency (Morice, 1996). The same is likely to be true for measuring reflexive cough thresholds. Table 3 summarises some of evidence for within-subject variability in cough reflex thresholds. A large number of studies have found cough reflex thresholds to be highly correlated within subjects, with variations rarely more than one dose, or one "doubling dose" above or below initial thresholds. However, such a statement does not take into account that the difference in concentration of tussive agent between

Table 3.

Evidence of Within-subject Variability in Cough Reflex Testing

Fujimura, Sakamoto, Kamio, & Matsuda (1992)	
Study population	Healthy adults (*N* = 38)
Nebuliser type	Bennett Twin nebuliser
Inhalation method	Tidal breathing, 15 seconds
Tussive agent	Tartaric acid 1.56 – 800 mg/mL
Outcome measure	C_5 response
Reproducibility testing	Test repeated within three days in a subgroup of nine participants
Reproducibility of outcome measure	Reproducible – no correlation between the difference and size of thresholds.
Comments	Difference in thresholds was within two doubling concentrations.
O'Connell, Thomas, Studham, Pride, & Fuller (1996)	
Study population	Healthy adults (*N* = 103)
Nebuliser type	Dosimeter-controlled nebuliser

Inhalation method	Vital-capacity method, one-second inhalation
Tussive agent	Capsaicin 0.5-500 μM
Outcome measure	Number of coughs in one minute
Reproducibility testing	A control group (*n* = 26) were measured at 6-18 months after baseline test
Reproducibility of outcome measure	Reproducible – 95 % of thresholds were reproducible within one incremental dose
Pounsford & Saunders (1985)	
Study population	Healthy adults (*n* = 8)
Nebuliser type	Wright's nebuliser, 10 L/minute
Inhalation method	Vital capacity method, five second inhalation
Tussive agent	Citric acid 0.025 – 0.9 M
Outcome measure	Presence of cough
Reproducibility testing	Tested over two morning sessions and two afternoon sessions
Reproducibility of outcome measure	Reproducible

Comments	No more than two doses difference between morning and afternoon. A non-significant trend for cough reflex thresholds to decrease over time.	
Barber et al. (2005)		
Study population	Healthy adults (N = 25)	
Nebuliser type	Hand-held glass DeVilbiss 40 nebuliser	Air-driven nebuliser controlled by a Mefar MB3 breath-activated dosimeter
Inhalation method	Vital-capacity method, two-second inhalation	Vital-capacity method, one-second inhalation
Tussive agent	Citric acid 0.01 – 3 M	Citric acid 0.01 – 3 M
Outcome measure	Number of coughs in 10 seconds	Number of coughs in 10 seconds
Reproducibility testing	Test repeated at the same time of day on consecutive days in 11 participants	Cough thresholds compared to alternative nebuliser
Reproducibility of outcome measure	Reproducible – r = 0.96. 91 % of thresholds were reproducible within one incremental dose	Reproducible – outcomes measured by the different nebulisers correlated highly, r = 0.95

Comments	Measurements obtained using two different nebulisers correlate highly, but differ by 2-3 threshold levels	
Di Franco et al. (2001)		
Study population	Healthy adults (n = 16)	
Nebuliser type	DeVilbiss 646 jet nebuliser	
Inhalation method	Vital-capacity method, one-second inhalation	
Tussive agent	Citric acid 0.5 – 10^{-4} M	Capsaicin 10^{-4} – 10^{-8} M
Outcome measure	Number of coughs in one minute	Number of coughs in one minute
Reproducibility testing	Test repeated after one week in a subgroup of eleven participants	Test repeated after one week in a subgroup of nine participants
Reproducibility of outcome measure	Reproducible – ICC = 0.75	Less reproducible – ICC = 0.55
Dicpinigaitis (2003b)		
Study population	Healthy adults (N = 80)	
Nebuliser type	Compressed air-driven nebuliser (DeVilbiss 646), dosimeter-controlled, 1 mL/minute	

Inhalation method	Vital-capacity, one-second inhalation
Tussive agent	Capsaicin 0.98 - 1000 μM
Outcome measure	C_2 and C_5 response in 15 seconds
Reproducibility testing	Forty participants measured two weeks after initial testing. Forty participants measured 6-62 months after initial testing
Reproducibility of outcome measure	Reproducible – 75-85 % of short-term thresholds and 83 % of long-term thresholds were reproducible within one doubling dose
Comments	Short-term reproducibility was superior to long-term reproducibility

Rees & Clark (1983)

Study population	Healthy young males (N = 8)
Nebuliser type	Hudson nebuliser
Inhalation method	Vital capacity method, one- second submaximal inhalation
Tussive agent	Citric acid 1 – 50 %
Outcome measure	Single cough

Reproducibility testing	Tested three times at the same time of day. Tests at least one week apart
Reproducibility of outcome measure	Reproducible – cough thresholds never varied by more than one dose level

Bickerman et al. (1954)

Study population	Healthy adults (*n* = 21) and adults with asthma (*n* = 17)
Nebuliser type	Vaponefrin nebuliser, flow rate 6 L/minute
Inhalation method	Five successive inhalations of tussive agent
Tussive agent	Citric acid 5 – 10 %
Outcome measure	Number of coughs during in five minutes
Reproducibility testing	Tested at weekly or monthly intervals over 2 – 9 months. The number of tests varied between participants
Reproducibility of outcome measure	Undetermined
Comments	Descriptive statistics reported only. Participants' responses were "fairly consistent" (p. 162)

Midgren, Hansson, Karlsson, Simonsson, & Persson (1992)

Study population	Healthy adults (*N* = 26)

Nebuliser type	Bird asmastick, flow rate 0.5 mL/minute	
Inhalation method	Tidal breathing method, tussive presented for one minute	
Tussive agent	Capsaicin 2, 10, 50 μM	
Outcome measure	Number of coughs during presentation of tussive	
Reproducibility testing	Re-tested at 20 minutes and 1-14 days following initial test (n = 12)	Re-tested three months following initial test (n = 9)
Reproducibility of outcome measure	Reproducible for 10 and 50 μM ($p > .05$) but not for 2 μM ($p < .05$)	Reproducible for 2 and 10 μM ($p > .05$). 50 μM not tested

Nejla, Fujimura, & Kamio, 2000

Study population	Healthy adults (n = 22) and patients with respiratory illness (n = 4)	
Nebuliser type	Dosimeter-controlled jet nebuliser (DeVilbiss 601)	Bennett Twin jet nebuliser, 5 L/minute
Inhalation method	Single inhalation	Tidal breathing for fifteen seconds
Tussive agent	Capsaicin 0.49 – 4000 μM	Capsaicin 0.49 – 4000 μM

Outcome measure	C_5 during one minute period following presentation of tussive	C_5 during presentation of tussive
Reproducibility testing	Test repeated one – three weeks following initial test	
Reproducibility of outcome measure	Reproducible – $r = 0.76$	Reproducible – $r = 0.84$

doses can often be large. Importantly, it is unknown whether such differences are clinically significant. For example, the difference between a cough reflex threshold of 0.4 mol/L and 0.6 mol/L citric acid is enough to accurately distinguish a silent aspirator from non-silent aspirator in a hospital setting (Miles et al., 2013b). It is difficult to draw firm conclusions from the literature as few studies have controlled for the potentially confounding variable of time of day on cough response. There are also large within- and across-study variations in the amount of time between repeated measures as well as tussive agent, nebuliser and tussive dose. Information regarding the instructions given to participants is rarely reported and there is a lack of consistency in the method of outcome measurement. In 2007, Morice et al. called for the amount of information about CRT reproducibility to be increased. To date, this has yet to be adequately addressed.

4.2.11. Drug effects on reflexive cough.

Several medications have been documented to either suppress or enhance reflexive cough sensitivity. Opioids have been shown to suppress the reflexive cough response in animal models (Kamei, 1996) and to reduce suppressed cough reflex sensitivity in healthy human participants (Kelly, Shaw, Brett, Greenwood, & Huckabee, 2016). Angiotensin-converting enzyme (ACE) inhibiting drugs are commonly used to treat hypertension and have also been shown to increase cough reflex sensitivity in animal models (Ebihara, Sekizawa, Ohrui, Nakazawa, & Sasaki, 1996; Sekizawa et al., 1996). Antihistamines may also reduce cough reflex sensitivity in patients with chronic cough, but do not affect the cough reflex sensitivity of healthy people (Tanaka, Hirata, Kurihara, Yoshikawa, & Takeda, 1996). From a clinical perspective, the various interactions between medication and cough reflex sensitivity may be less relevant as the outcome of interest is simply the patient's risk of silent aspiration.

4.2.12. Medical conditions and their relationship to the cough reflex.

Several conditions have been documented to result in increased cough reflex sensitivity, including gastroesophageal reflux disease (Phua, McGarvey, Ngu, & Ing, 2010), asthma, (Koskela, Purokivi, Kontra, Taivainen, & Tukiainen, 2008), allergic rhinitis (Pecova, Vrlik, & Tartar, 2005), atopic dermatitis (Pecova, Frlickova, Pec, & Tatar, 2003) and chronic obstructive pulmonary disease (COPD [Doherty, Mister, Pearson, & Calverley, 2000]). While smoking reduces cough reflex sensitivity (Dicpinigaitis, 2003a), smoking cessation leads to improved reflexive cough sensitivity after only two weeks (Dicpinigaitis et al., 2006). It is unknown whether this enhanced cough reflex sensitivity may be present earlier than two weeks post-smoking cessation. A recent study demonstrated that a reflexive cough sensitivity is significantly inhibited after only a single electronic cigarette (e-cigarette) session (Dicpinigaitis, Chang, Dicpinigaitis, & Negassa, 2016). In this study, the effects of the e-cigarette were transient (i.e. lasting less than 24 hours) and only observed in e-cigarettes containing nicotine.

Decreased cough reflexive sensitivity has been documented in patients with pneumonia in two small studies. Niimi et al. (2003) measured cough reflex sensitivity to inhaled capsaicin in seven patients with recurrent pneumonia and no underlying medical condition and compared this to healthy age- and gender-matched controls. Cough reflex thresholds were significantly higher in the recurrent pneumonia group compared to controls. Nakazawa, Sekizawa, Ujiie, Sasaki and Takishima (1993) measured cough reflex sensitivity to inhaled citric acid in ten patients with dementia, ten patients with aspiration pneumonia and ten healthy controls. Participants were closely age-matched. While the control participants had an average cough reflex threshold of 2.6 mg/mL, cough thresholds were higher in participants with dementia

and aspiration pneumonia, at 37 mg/mL and 180 mg/mL, respectively. Seven out of ten participants with aspiration pneumonia did not cough at the highest level of citric acid, 360 mg/mL. Unfortunately no further statistical analyses or instrumental swallowing assessments were undertaken so it is not clear how statistically or clinically relevant these differences were. The authors also did not provide further details about how aspiration pneumonia was diagnosed or how recent the diagnosis was, which makes it difficult to generalise these findings further.

The studies presented by Niimi et al. (2003) and Nakazawa et al. (1993) raise an interesting question: does decreased cough reflex sensitivity lead to pneumonia or does pneumonia decrease cough reflex sensitivity? Further investigations involving larger sample sizes are required to definitely answer this question. However, other explanations must also be considered, such as the relationship between oral hygiene and reflexive cough sensitivity in patients with recurrent pneumonia.

In patients with acute neurological injury such as stroke, the transient or permanent loss of reflexive cough sensitivity has been related to a condition referred to as 'brainstem shock' (Addington, Stephens, Widdicombe, & Rekab, 2005). This condition is defined as a global neurological event affecting one or more vital functions, including the reticular activating system, respiration and the cough reflex. Patients in brainstem shock may have reduced consciousness, reduced respiratory drive and/or reduced cough reflex sensitivity, irrespective of stroke location. Addington et al. propose that recovery from brainstem shock may be an important predictor of patient outcomes.

Others have proposed that certain neurological injuries may actually heighten cough reflex sensitivity. Smith and Wiles (1998) proposed a model whereby corticobulbar pyramidal tract lesions may lead to hypersensitivity of the cough reflex.

This theory was based on their study of 28 heterogeneous patients on a neurological ward. They reported that patients with dysphagia (as identified by a water-swallowing test) had similar or increased cough reflex sensitivity to inhaled capsaicin as compared with non-dysphagic patients. This study was significantly limited by the lack of a homogeneous patient cohort, lack of instrumental assessment for dysphagia, small sample size and lack of normative data for capsaicin-induced cough thresholds and the extent to which results can be generalised to typical patients with acute stroke is uncertain. It remains unknown whether patients with acute stroke can be categorised as either hypo- or hyper-reflexive in terms of cough sensitivity by their specific site of lesion. Further investigation into patterns of recovery of the cough reflex in stroke is also warranted.

While CRT provides clinicians with insight into patients' risk of silent aspiration, in order to ensure positive outcomes for patients, CRT results must be incorporated appropriately into clinical management decisions. One way to achieve this may be the use of standardised management protocols.

4.3. Standardised Patient Management

In their review of healthcare quality, Every et al. (2000) explain the difference between clinical guidelines, clinical protocols, and clinical pathways. Clinical guidelines are recommendations developed to assist clinicians and patients in choosing the appropriate course of action in response to a specific condition or scenario. Guidelines incorporate expert consensus and current scientific knowledge relating to the prevention, diagnosis, treatment, and management of particular diseases (Hasnain-Wynia, 2006). Clinical pathways, while based on clinical guidelines, are management plans that describe patient goals as well as the timing and sequence of events required to achieve these goals with maximum efficiency. Such

pathways are useful tools that can highlight inefficiencies in health services irrespective of whether there is evidence for implementing changes to those services. There are certain benefits of clinical pathways, for example, as a means of reducing variation in patient care, improving quality of care, and maximising resource usage. Similarly, clinical protocols are intended to reduce variation in patient care. However, unlike clinical pathways, clinical protocols place more value on guideline compliance than identifying opportunities to streamline treatment. They are treatment plans or specific steps to be taken in patient care, and the patient's response is continuously monitored. Protocols can be conceptualised as a practical, step-by-step guide to implementing clinical guidelines (Hasnain-Wynia).

Although it may seem intuitive that flexibility in decision-making regarding an individual patient's presentation may be beneficial to that patient's care, the opposite may in fact be true: patients who are managed with standardised protocols have better outcomes compared to patients receiving non-standardised care. An example of this can be found in the field of acute traumatic brain injury (TBI), a population whose treatment has traditionally been highly variable between facilities and clinicians. Fakhry, Trask, Waller and Watts (2004) devised a standardised protocol for all patient with severe TBI that incorporated staff education and the timing of medications and specific interventions. After 6 years, compliance with the protocol was 85 %. Compared to pre-protocol figures, patients managed with the new protocol spent significantly less time in the intensive care unit and total length of hospital stay was reduced by five days. In addition, the overall mortality rate dropped by 4 % and patient outcomes (as measured by the Glasgow Outcome Scale) significantly improved to the point where the over half of patients experienced either "good recovery" or only "moderate disability". As compliance with the protocol improved,

patient mortality and length of stay outcomes also improved. The authors concluded that eliminating variance in treatment using a standard care protocol was effective at improving patient outcomes and reducing the associated cost of care.

Although compelling, the findings from this study are not immune to the significant effects of bias, due to the prospective-retrospective nature of this study. The possibility that random variation, or other uncontrolled factors, played a part in the results did not go unrecognised by the authors. Although a positive trend towards increased survival was observed, the causes of death in both the historical and present-day cohorts were not reported, leaving the reader to question the reason for increased survival rates. Could it be possible that improvements in survival were due to factors unrelated to TBI? Without additional details, it is impossible to tell. Similarly, it is difficult to draw strong conclusions from the authors' report of reduced length of hospital stay. Such an outcome is sensitive to changes in policy, inter-facility transfer agreements, numbers of beds, funding, etc, and as such the results should be interpreted with caution.

Similar reports of success following the implementation of standardised patient management protocols have been reported in more recent studies among patients with a range of morbidities from septic shock (Micek et al., 2006), fractured neck of femur (Friedman, Mendelson, Kates, & McCann, 2008) and oesophageal cancer (Low et al., 2007). Despite this, the literature surrounding standardised management in patients with dysphagia is surprisingly scarce; research regarding dysphagia post-stroke is even more rare.

4.3.1. Dysphagia assessment and management protocols in acute stroke care

During the acute phase, patients with stroke have a very high risk of dysphagia, pneumonia, and mortality from aspiration pneumonia (Addington, Stephens, & Gilliland, 1999; Holas et al., 1994; Martino et al., 2005) and standardisation of patient care following stroke is strongly supported as a means of improving patient outcomes (Altman, 2011).

International published guidelines from both the American Stroke Association (Jauch et al., 2013) and the Stroke Foundation of New Zealand (Stroke Foundation of New Zealand & New Zealand Guidelines Group, 2010) state that all acute stroke patients should remain NBM until they have undergone swallowing screening and be referred for SLT dysphagia assessment if indicated. Yet despite these few very clear recommendations, details regarding dysphagia assessment and consequent management are distinctly lacking.

Historically, research in this field has focussed on the use of dysphagia screening protocols (e.g. Hinchey et al., 2005; Perry & McLaren, 2003) and standardisation of dysphagia assessment tools (e.g. Mann & Hankey, 2001; Middleton et al., 2011), while protocols for the initiation of oral feeding and prescription of diet consistency are lacking. In fact, only six studies have been identified which have attempted to evaluate the effects of a dysphagia assessment and management protocol in acute stroke care. One of these, conducted by Addington et al. (1999), has already been discussed above.

One of the first published reports of a dysphagia-specific stroke management protocol was by Odderson et al. (Odderson et al., 1995), who studied 124 patients with acute non-haemorrhagic stroke who were managed according to a dysphagia

assessment and management protocol. The assessment arm of the protocol consisted of dysphagia screening conducted by a SLT or nurse. Screening items included the patient's level of alertness, a subjective judgement of voice and volitional cough strength, the ability to swallow water and ice chips 'briskly', subjective evidence of laryngeal elevation during swallowing and no overt signs of post-swallow aspiration. Patients who passed screening proceeded to an oral diet of puree consistency with thin and thick fluids; if this was tolerated the patient could advance to other textures. Patients who failed screening remained on a restricted diet and were referred for instrumental swallowing assessment. All patients were reviewed daily by a SLT. A total of 39 % of patients failed swallowing screening and were considered to be dysphagic. In the year-long study period, no patients developed AP. Three patients died, however the cause of death was not reported. Unfortunately, no comparisons were drawn between patients managed per the dysphagia protocol and a control group of patients not managed per a protocol. The authors reported that the use of this protocol was successful and cost-effective, although this is difficult for the reader to judge in the absence of a control group. The rationale and implementation of this protocol is problematic for several reasons. First, the authors' justification for using ice chips/water in the swallowing screen was that aspiration occurs only on liquid boluses. This is inaccurate. Second, the presence of a strong volitional cough was included in the screen to identify patients at risk of aspiration. It is now known that volitional coughing activates different neural networks from reflexive coughing (Mazzone et al., 2011) and results in a different expiratory pattern (Addington, Stephens, Phelipa, Widdicombe, & Ockey, 2008). For these reasons, Addington et al. (2008) have stated that it is inappropriate to attempt to assess reflexive airway protection status using voluntary cough measures. Third, the screening items do not

account for silent aspiration. Fourth, even patients who failed screening were provided with oral intake, although the compensatory strategy of diet modification was put in place. Given these limitations, it is surprising that no patients developed AP during the year-long study period. Perhaps other aspects of stroke management, e.g. aggressive antibiotic use, could account for this finding.

Leder, Suiter, Warner, Acton, and Siegel (2012), investigated the effects of a dysphagia assessment and management protocol in a heterogenous group of patients (including stroke) with suspected dysphagia who were referred for a SLT assessment of swallowing. Participants (N = 1000) were recruited from a tertiary care, teaching hospital. Participants had a range of aetiologies including head and neck surgery, dementia, Parkinson's disease and stroke. Details of pre-existing medical conditions were not reported. The protocol dictated that all patients underwent a 90 mL water swallow challenge (uninterrupted drinking from a cup or using a straw). An inability to drink the entire amount uninterrupted or coughing during or immediately following the test were considered a 'fail' response; these patients were excluded from the rest of the study. If a patient passed the water swallow challenge, the SLT completed an oromotor examination and a basic cognitive bedside evaluation. Diet recommendations were based on these results: if the patient was edentulous, they received a puree diet and regular fluids, all other patients received either a puree, soft or regular diet with regular fluids. Patients received a follow-up visit from the SLT within 12-24 hours and nursing staff discontinued oral intake if overt signs of aspiration were noted. Outcomes were measured via chart review after 1 day. Outcomes of interest were: diet level, percent of meal eaten and volume of liquid ingested during a meal. Ninety-three patients were excluded from analysis after their medical condition worsened post-swallowing assessment. No further details about

these patients were reported so it cannot be ruled out that worsening pulmonary function may have been present. Results revealed that all patients were drinking regular fluids "successfully and safely" at 12-24 hours post-swallowing assessment, although the criteria for determining this was not reported. Patients ate between 10-100 % of their first meal, however, more specific data for this outcome was not reported, nor was the proportion of the different diet consistency levels. The authors concluded that the swallowing assessment and management protocol assisted SLTs to recommend specific oral diets successfully, negating the need for instrumental assessment of dysphagia among patients who pass the 90 mL water swallowing challenge.

While the authors should be commended for tackling an important, under-researched aspect of dysphagia assessment and management, the methodology employed in this study unfortunately limits the usefulness of results. For example, patient outcomes were only evaluated at one point in time, 12-24 hours after swallowing assessment. By neglecting to measure patient outcomes beyond this time period, the opportunity to investigate important long-term outcomes was lost. Hospital-acquired pneumonia is defined as pneumonia which develops at least 48 hours following admission (American Thoracic Society, 2005) and is easily measured by employing the same chart review technique that was used in this study. In addition, the validity of the outcome measures is questionable. 'Total amount ingested' and 'diet recommendation' are only two potential outcomes of dysphagia assessment, while other important outcomes such as penetration/aspiration, pneumonia, nutritional status, quality of life, and mortality were overlooked. Based on their limited outcome measures, the authors have over-generalised their results to conclude that 100 % of patients were able to swallow "successfully and safely" post-assessment. One could

argue whether the patients (*n* = not reported) who only ingested 10 % of their first meal were indeed swallowing "successfully" and an absence of long-term health outcome measurement (e.g. aspiration pneumonia) and instrumental assessment limits the extent to which comments can be made about swallowing "safety".

Also of concern is the apparent lack of consideration of silent aspiration. The main author has also published a study that reports silent aspiration as 'volume dependent' i.e. individuals who silently aspirate small bolus volumes (i.e. 1-10 cc) tend to show overt signs of aspiration on larger bolus volumes [i.e. 90 mL] (Leder, Suiter, & Green, 2011). In fact, what the 2011 study showed was that over 50 % of small-volume, liquid silent aspirators did not cough while completing the 90 mL water-swallow test. On this basis, the authors' conclusion that the issue of silent aspiration is mitigated when using the 90 cc water-swallow test is questionable.

Although the study population was both large and heterogenous, the greatest proportion (36 %) of patients had presented to hospital with 'medical' aetiologies. It is unclear whether such heterogeneity increases generalizability to patients outside of this study or whether it disguises any effects specific to sub-populations, such as acute stroke. The authors identified that this protocol is not appropriate for use with tracheostomised patients due to the high risk of silent aspiration in this group and likelihood of high false negative results.

This study assessed a protocol for dysphagia assessment and management in the acute setting. Further development of such a tool must include a plan for rehabilitation and review if SLTs are to continue to offer a holistic approach to patient care. Burek et al. (2008) conducted a three-year study documenting outcomes relating to pneumonia in patients with stroke following the implementation of a strict acute clinical management protocol. The protocol included nurse dysphagia screening

within two hours of admission to hospital, standardising dysphagia assessment and intervention provided by SLTs and optimising VFSS imaging protocols. Details of SLT dysphagia assessment and interventions were not reported. It was mentioned that when SLT services were not available (i.e. after hours), the Standardized Swallowing Assessment (Perry, 2001) was performed, although details of who performed this were not provided. An additional focus of this study was on education of the MDT. For example, nurses were provided training in oral hygiene and assisting patients with eating. This training occurred in the first year of the study only.

A significant overall reduction in pneumonia rates was documented from 11.1 % to 5.4 % over three years following implementation of the clinical management pathway. The proportion of patients with dysphagia changed minimally from year to year, as did the distribution of dysphagia severity levels. Interestingly, among patients who were considered severely dysphagic, the rate of pneumonia decreased dramatically from 37 % to 21 % in the first year of the protocol. However, in the second year, the pneumonia rate rose to 47 %. The authors speculated that the reason for this may be that those patients were simply too severely impaired to benefit from the protocol, which may be better suited to patients with mild to moderate dysphagia. On the other hand, it should be noted that patients who were identified as dysphagic during initial screening were not made NBM until a VFSS was performed three to four days later. It is possible that recommending NBM earlier may have resulted in fewer incidences of pneumonia.

It may also be argued that this protocol failed to address the issue of silent aspiration by including the Standardized Swallowing Assessment (Perry, 2001), which is based almost entirely on observations of water-swallowing and has questionable validity, sensitivity and specificity. Horner and Massey (1988) found a

silent aspiration rate of 38 % in patients who were, on average, 2.8 months post-stroke. This rate may be much higher in acute stroke patients, and as such, a swallowing assessment that relies on the patient having an observable cough response may be expected to yield very high false negative rates. Despite the above limitations, Burek and colleagues (2009) did provide promising evidence in support of standardising acute dysphagia management. Regrettably, by omitting the details of SLT intervention, the extent to which the study can be replicated, or the intervention can be applied in real-world settings, is severely limited.

Gandolfi et al. (2014) retrospectively evaluated a multidisciplinary management protocol in patients with post-stroke dysphagia (n = 39) compared to a control group receiving usual care (n = 45) over a four-year period. A neurologist assessed potential participants for clinical signs of dysphagia using a water-swallowing test and those with no evidence of dysphagia were excluded. Pre-existing dysphagia was also an exclusion criterion for this study. All participants underwent a clinical assessment of swallowing including cranial nerve examination and water-swallowing tasks as well as either a VEES or VFSS. Following instrumental evaluation, participants received five hours of swallowing rehabilitation per week until discharge. Swallowing rehabilitation involved 'sensorial stimulation', facial and mouth exercises, breathing exercises, implementing compensatory strategies and oral trials. No further details of these rehabilitation strategies were reported and no specific exercise or stimulation protocols were cited. Outcome measures included mortality, occurrence of pneumonia, need for respiratory support and need for nutritional support at discharge. Pneumonia was defined as the occurrence of three or more of the following symptoms: fever >38 °C), abnormal chest x-ray, productive cough and purulent sputum, abnormal respiratory examination and need for

antibiotics. There were no differences in demographic variables between the group who received the protocol and the control group. Patients who were managed using the protocol had a significantly lower risk of needing respiratory support during hospitalisation compared to the control group, however there were no differences in the rates mortality or tube-feeding at discharge. While patients managed under the protocol had a significantly lower risk of pneumonia compared to control patients, there were no differences in the frequency of pneumonia between the two groups. Given these results, it is surprising that the authors drew firm conclusions that the use of a protocol can significantly reduce aspiration pneumonia and in-patient mortality.

Methodological limitations make it difficult to ascertain the true value of the protocol described by Gandolfi et al. (2014). Specifically, by screening potential participants for dysphagia on the basis of a clinical evaluation and water-swallowing test creates a selection bias against patients with silent aspiration. Although this bias would have been present for both the treatment and control groups, it limits the generalizability of findings to patients with overt aspiration only. The rehabilitation aspect of the protocol is also significantly flawed. Although the actual numbers of participants who received VEES versus VFSS was not reported, providing dysphagia rehabilitation based on a VEES is inappropriate as this method does not provide information about the oral phase of swallowing and does not allow detailed analysis of pharyngeal kinematics. Limited information about the rehabilitation provided to participants in either study group was reported with no direct referencing of evidence-based techniques. It is unknown how many hours of rehabilitation the control group received and it is likely that this represents a confounding variable. It is also problematic that pneumonia outcomes were only analysed amongst surviving participants. Although the authors reported that no participants in the protocol group

died from pneumonia, including participants from the control group who died from pneumonia would have been informative and potentially altered the results. Finally, it seems that future studies of this nature must include a larger sample population as the authors noted they had low statistical power (1 - β not reported).

Ickenstein et al. (2010) evaluated a dysphagia protocol in patients with acute stroke ($N = 114$). Every patient received a nurse-administered dysphagia screening on day one of admission. The screening involved observations about the patient's secretion management, alertness, vocal quality and tongue movement. If there were no concerns, the patient proceeded to a water-swallowing challenge with one teaspoon of water. According to criteria published by Perry (2001), if there was no evidence of swallowing, if anterior leakage, coughing or a change in respiratory rate or vocal quality were present the test was terminated, or if the nurse suspected that dysphagia was present. The patient then proceeded to the 90 mL water-swallowing test (Suiter & Leder, 2008). As per the test criteria, if coughing or a change in vocal quality was observed or if the test was unable to be completed then screening was terminated. Depending on the results of screening, the patient proceeded to a 'swallowing-adjusted diet', which ranged from normal to NBM. Decisions about adjusting diet consistencies were made in conjunction with the SLT or doctor. Patients deemed an aspiration risk were recommended NBM and patients with mild to moderate difficulties were recommended an altered texture diet. In conjunction with this screening, patients received a clinical swallowing evaluation from a SLT within 72 hours of admission to hospital and a VEES within five days. Specific details of these examinations were not reported. Patients then received rehabilitation from the multidisciplinary team, although specific details were not reported. Outcomes were measured by comparing results to retrospective data from preceding years and to

other hospitals in the area. The main outcomes were mortality and pneumonia, although the authors do not mention how pneumonia was diagnosed. Outcomes relating to mortality were analysed descriptively. Following the implementation of the protocol, mortality rates reduced from 7.4 % to 4.2 %. Pneumonia rates reduced from 8 % to 2.8 %, significantly lower than the 9 % reported from surrounding hospitals. As no patient demographic or clinical variables were reported, the extent to which results can be generalised outside of this study is limited. It is also unclear whether any of these variables were taken into account during statistical analysis (i.e. logistic regression), which could be considered essential when drawing comparisons between prospectively- and retrospectively-collected data. It seems as though the authors' aim in conducting this study was to reduce the time between a patient's admission to hospital and initiation of dysphagia management by incorporating nurse-led dysphagia screening. Patients with no overt signs of dysphagia could commence a normal oral diet without delay while those with signs of dysphagia could be placed on a 'safer' oral diet or alternative route of nutrition until a formal swallowing evaluation was completed. Whether it is appropriate for patients who are considered mildly to moderately dysphagic to be given an oral diet prior to an instrumental evaluation of swallowing is debateable. It also seems unlikely that this particular screening method – which relies on patients displaying overt signs of dysphagia – is sensitive enough to detect patients with silent aspiration.

4.4. Summary

The cough reflex is a dynamic physiologic response (Dicpinigaitis, 2007). Under certain conditions a person may show a different pattern of cough reflex sensitivity. For example, people with dementia (Nakazawa et al., 1993) or chronic chest infections (Niimi et al., 2003), smokers (Dicpinigaitis, 2003a) or those on opioids

(Kelly et al., 2016) may have reduced cough reflex sensitivity, while people on ACE inhibitor drugs, who have chronic coughs or have recently stopped smoking tend to have heightened cough reflex sensitivity. Time of day and familiarity with the CRT may also play a role. Although an individual's cough reflex is subject to multiple influences, there appears to be a clinically relevant threshold for cough sensitivity, which, if not triggered, may result in silent aspiration (Miles et al., 2013b).

While various dysphagia screening and assessment protocols have been assessed for their ability to identify patients at risk of aspiration, a common limitation is the lack of consideration of silent aspiration. The ability to identify patients at risk of silent aspiration on a clinical evaluation would greatly enhance the clinician's ability to make appropriate recommendations and would presumably improve patient outcomes. CRT may be an ideal way of assessing silent aspiration risk and has been validated against instrumental assessment with acceptable sensitivity and specificity (Miles et al., 2013b). Despite this, the use of CRT in a standardised dysphagia assessment and management protocol has received little attention in the dysphagia literature. Studies to date have been plagued by methodological limitations and the range of CRT methods used makes comparisons difficult. Further research into this aspect of acute stroke care is required.

Citric acid may be an ideal choice of tussive agent as it stimulates not only the chemoreceptors of the larynx but also the mechanoreceptors and it is a well-established and safe technique with established normative data. In an acute stroke population, the tidal breathing method may avoid some of the difficulties that come with using a mouth-tube. The trade-off may be increased within-subject variability in the dose received due to variation in respiratory rates.

The presence of oral bacteria is also strongly aligned with adverse pulmonary consequences. Protocols that reduce oral bacterial loading and colonisation of pathogenic microorganisms reduce the risk of pneumonia (Simmons-Trau et al., 2004; Bousbia et al., 2012; Yoneyama et al., 2002; Yoshino et al., 2001; Watando et al., 2002). This reduction in pneumonia can easily be attributed to a reduction in oral pathogens. However, Watando et al. (2002) identified another direct effect of oral hygiene: improved cough reflex sensitivity. This suggests that intensive oral care can increase airway protective mechanisms. However, it remains unknown whether improved cough reflex sensitivity is due to decreased oral bacterial load, increased mechanical stimulation of the oral cavity or a combination of both. Clarification through further investigation is required if we are to provide appropriate interventions for patients at risk of AP. Furthermore, despite a known increase in oral carriage of yeasts in acute stroke, there are no specific studies documenting the relationship between oral bacteria, cough sensitivity and pneumonia in the acute stroke population. Given that this population is at substantial risk of both aspiration and consequent pneumonia, this represents a significant gap in the knowledge base supporting clinical care.

Chapter 5. The Role of Bacteria in Aspiration Pneumonia

The presence of potential respiratory pathogens in the oral cavity is recognised as a significant factor in the pathogenesis of pneumonia (Langmore et al., 1998; Scannapieco, 2006). The oral cavity is in constant contact with environmental pathogens when breathing, eating contaminated food and being exposed to contaminated hands or equipment. There are many surfaces present in the oral cavity on which potential respiratory pathogens may colonise. Subsequent aspiration of these pathogens increases the risk of developing a chest infection (Langmore et al., 1998; Scannapieco, 2006). Understanding the role of oral bacteria in AP may assist in predicting and preventing this disease.

5.1. Oral Microflora

In most people, there is a dynamic equilibrium between the oral microbial community and the innate defence systems of the body. Bacterial flora is the most common oral flora, with over 500 different strains present in the oral cavity, but fungi, protozoa, and occasionally, viruses, may also be present (Nagoba & Nagoba, 2007). Although oral bacteria can assist in the digestive process, their main role seems to be in the prevention of disease. The presence of a resident microbe community, or "core microbiome" (Zaura, Keijser, Huse, & Crielaard, 2009, p. 2), may serve to reduce the risk of colonisation by pathogenic microbes via the process of natural selection (Marsh, 2000).

5.1.1. Identification and classification of bacteria.

By convention, bacteria are referred to in terms of composition of the cell wall (Gram-classification) and oxygen requirements. The Gram test involves staining the cell wall with a crystal violet dye. Bacteria with a thick, single-cell layer wall will retain a purple colour and are classified as Gram-positive. Bacteria with a cell wall

more than one cell thick will not retain the purple stain and are classified as Gram-negative. Aerobic bacteria are oxygen-dependent, anaerobic bacteria cannot survive in the presence of oxygen and facultative, or aerotolerant, anaerobic bacteria can live with or without oxygen. Aerobic bacteria are rare among the indigenous oral flora (Socransky et al., 1963) with the vast majority being facultative anaerobic. The role of Gram-negative, Gram-positive, aerobic and anaerobic bacteria in AP is debated in the literature and is discussed in depth in a later section.

In healthy mouths, innocuous bacteria act as a barrier against colonisation by respiratory pathogens (Jenkinson & Lamont, 2005). Poor oral health may select against these bacteria, altering the balance from mainly Gram-positive bacteria to mainly Gram-negative bacteria (Sbordone & Bortolaia, 2003). Gram-negative, anaerobic bacteria are often pathogenic, can easily colonise oral surfaces, reproduce in large numbers and spread throughout the oral cavity via saliva (Pace & McCullough, 2010). The presence of oral pathogens has been suggested as a necessary precursor to aspiration pneumonia (Langmore et al., 1998). Therefore, testing the saliva for the presence of such pathogens may be one way of determining pneumonia risk.

5.1.2. Flora of the normal oral cavity.

Before exploring the oral microbial profiles associated with disease, it is pertinent to understand the oral microbial composition of a normal mouth. The oral microbiome can be considered an umbrella term that encompasses each of the distinct sites in the mouth (tongue, cheeks, hard and soft palate, tonsils, dental biofilms). Saliva, which washes over each of these sites, contains approximately 100 million bacterial cells per millilitre (Curtis, Zenobia, & Darveau, 2011).

The oral microbiome has been the subject of thorough investigation through established links between oral bacteria and disease in humans. As such, it is the most completely-characterised microbiota in humans (Curtis et al., 2011). By extracting and culturing tissue samples, early investigations have successfully identified differences in the types and quantities of microbes from different areas of the oral cavity, reflecting the different environments provided by the teeth, tongue, gingival crevices and saliva (e.g. Gibbons, Socransky, de Araujo, & van Houte, 1964; Gibbons, Socransky, Sawyer, Kapsimalis, & Macdonald, 1963; Gordon & Gibbons, 1966; Gordon & Jong, 1968; Richardson & Jones, 1958; Socransky & Manganiello, 1971). However, it has since been recognised that culture-based methods give an incomplete picture of the composition of the oral microbiome as a large proportion of oral bacteria cannot be cultured in laboratory conditions. Advances in culture-independent analysis techniques have led to the discovery of vast numbers of bacterial species that have yet to be cultured. In 2004, it was estimated that approximately half of all oral bacteria have not been cultured (Wade, 2004).

Using molecular-based analysis, Dewhirst et al. (2010) developed the Human Oral Microbiome Database (www.homd.org), a list of every species of bacteria found in the mouth. Eleven bacterial species are reliably present in the oral cavity: *Actinobacteria, Chloroflexi, Bacteroidetes, Firmicutes, Tenericutes, Spirochaetes, Fusobacteria, Synergistetes, Proteobacteria* and two unnamed phyla. Within each species are hundreds of individual species, representing the diversity of the human oral microbiome.

The term ‘commensal’ refers to a relationship in which one organism benefits and the other remains unaffected. Of interest, several respiratory commensal pathogens, while not considered to be part of the normal oral microbiome, can be

found in a percentage of healthy (i.e. asymptomatic) adults. For example, *E. coli* and *Klebsiella* spp. have been identified in the mouths of healthy volunteers (Mobbs, Van Saene, Sunderland, & Davies, 1999).

The oral carriage of commensal pathogens appears to be strongly linked to subsequent infection in 'at-risk' groups, such as hospitalised patients. This is discussed in further detail in the following section. In otherwise healthy adults, the reason that infection does not develop appears to be an intact ability to promptly clear these bacteria from the mouth, thus preventing colonisation.

5.1.3. Host factors that influence the oral microbiota.

Both pathogenic and innocuous bacteria can be found inside a healthy oral cavity, existing in a symbiotic relationship (Shiffer Nield-Gehrig & Willmann, 2007). Changes to the intrinsic host environment, such as those caused by acute or chronic illness, or change to oral hygiene routines, may disturb this equilibrium. There are many factors that may predispose individuals to oropharyngeal or tracheobronchial colonisation by pathogenic bacteria. Those most pertinent to stroke patients are discussed in detail below.

5.1.3.1. Age.

The composition of the oral microbiota is very heavily influenced by age. In the elderly, these differences are a result of various factors such as lowered immunity, illness, poor nutrition, the use of dental prostheses, salivary hypofunction, a diminished ability to perform oral hygiene routines independently and a lack of access to regular dental services (Carter et al., 2004; Wilson, 2005). Such changes lead to increased prevalence in the oropharynx of Gram-negative bacteria (e.g. *Klebsiella* spp., *E. coli* and *Enterobacter* spp.) but increased colonization of Staphylococci – a Gram-positive species – have also been reported (Wilson, 2005). Of note, elderly

individuals are also susceptible to diminished mucociliary function and loss of lung elasticity, which may lead to increased microbial colonisation of the respiratory tract as microbes that are normally expelled are able to remain (Skerrett, Niederman, & Fein, 1989; Wilson, 2005).

From as early as 1974, the link between oral bacteria and aspiration pneumonia among the elderly was established (Bartlett, Gorbach, & Finegold, 1974; Cesar, Gonzalez, & Calia, 1975; Lorber & Swenson, 1974). By the late 1990s, it had become clear that mortality and morbidity from pneumonia was high among dependent elderly (Scannapieco & Mylotte, 1996). However, the role of dentures and natural teeth as sites of pathogenic bacterial colonisation remained unknown. As the prevalence of denture use increases with age (Pietrokovski, Azuelos, Tau, & Mostavoy, 1995), an understanding of the relationship between dentition and oral bacteria was required in order to identify those at increased risk of AP.

Percival, Challacombe and Marsh (1991) were among the first to document age-related changes in oral bacteria. They investigated the presence and prevalence of select bacteria among healthy people aged 20-39 years (n = 30), 40-59 years (n = 23), 60-79 years (n = 16) and over 80 years (n = 10). Participants in this study were dentate (i.e. had at least seven teeth), did not use dental prostheses and were in good health (e.g. no oral disease or need for antibiotics). Saliva and plaque samples were collected and analysed for several target bacteria using both aerobic and anaerobic culture methods. Results revealed significant differences between the different age groups with regards to the prevalence of target bacteria. These included an increase in the prevalence of lactobacilli, staphylococci and *Actinomyces viscous* in saliva in people aged over 60 years compared to those aged 20-39 years. The prevalence of bacteria was especially high in people over 70 years. These results suggest that age-

related changes in the oral microbiota occur among healthy people. However, as only bacteria of "dental significance" were selected for analysis, the true extent of these age-related changes remains unknown. Generalisation of these results to the wider elderly population is limited, as the effects of denture-wearing and general health were not explored in this study. These factors are discussed in turn below.

5.1.3.2. Dentition.

There are several 'degrees of dentition', ranging from edentulous to fully dentate to dentate with the use of dental prostheses. The complete loss (or removal) of teeth may select against particular bacteria that colonise hard dental surfaces (Abdul-Kareem, 2012). For example, the *Neisseria* species can be found in newly-edentulous subjects but disappears after denture use, while *E. coli* and *K. pneumoniae* tend to be absent from edentulous mouths but can be found after one month of denture use (Abdul-Kareem, 2012). Binkley, Haugh, Kitchens, Wallace and Sessler (2009) reported that edentulous adults were more likely to be colonised with *S. pneumoniae* or *Methicillin-resistant Staphylococcus aureus* (MRSA) than dentate adults. However, these results were based on buccal mucosa samples from edentulous subjects and dental plaque samples from dentate subjects, which could account for the differences.

With the constant improvements and innovations in dental care, more people may be retaining their own teeth into old age. These same people may not necessarily be able to maintain adequate oral hygiene and thus may have increased numbers of diseased teeth (Pace & McCullough, 2010). Increased risk for infection may be a consequence of this.

Several studies have documented an increased prevalence of respiratory illness among dentate and edentulous elderly. Sumi et al. (2007) examined the dental plaque in a group of dependent elderly (N = 138, age range = 74 years). Although

information regarding comorbidities was not reported, all participants had evidence of poor oral hygiene, had not received antimicrobial drugs in the preceding eight weeks, had no history of diabetes and all were dependent on caregivers for oral hygiene. Sumi et al. reported that the presence of respiratory pathogens was 65 %. This number may in fact be an underestimation, as the sampling method described by Sumi et al. excluded anaerobic bacteria as well as bacteria that were unable to be cultured. The prevalence of respiratory pathogens reported by Sumi et al. is 1.5 times higher than a previous report from the same research group of pathogenic bacteria from dentures (Sumi et al., 2002). One reason for this difference may be that dentures can be removed and cleaned more thoroughly than fixed teeth.

In a study comparing dentate and edentulous participants, Mojon, Budtz-Jørgensen, Michel and Limeback (1997) reported that 40 % of dentate subjects had at least one incidence of respiratory illness compared to 27 % of edentulous subjects. In this study, participants also had a high overall rate of respiratory illness at 33 %. This rate may in fact have been under-reported, as the diagnosis of respiratory infection was made solely on the basis of clinical symptoms, which has low sensitivity and tends to result in false negative results (Woodhead, 2007). Yoneyama et al. (2002) reported that fewer edentate participants who received intensive oral hygiene developed pneumonia compared to dentate participants, although proportionately, the numbers were actually identical in both groups at 9 %. Among a no-oral-hygiene group, slightly fewer edentate participants developed pneumonia compared to dentate participants: 20 % and 21 %, respectively (Yoneyama et al., 2002). The studies by Mojon et al. and Yoneyama et al. suggest a link between dentition, oral health and respiratory health. This may be due to the fact that more hard surfaces are available for bacteria to colonise. However, in the absence of specific regression analyses, it is

not possible to determine whether dentition was a true independent predictor of pneumonia.

In 2006, Tachibana et al. (2006) used molecular analysis to document the prevalence of six oral pathogenic bacteria (*Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, *Treponema denticola* and *Streptococcus mutans*) in 85 year olds ($N = 204$). Few details about the participants were reported, however the mean number of teeth was provided ($\bar{x} = 7$). By using the Mann-Whitney *U*-test, the authors established significant differences between the number of teeth and the number of bacteria present. Those with greater than eight teeth (approximately) had higher levels of four out of six of the pathogenic bacteria compared to those with fewer than eight teeth. The use of molecular analysis techniques adds weight to this finding as this technique does not rely upon the cultivation of live bacteria. Unfortunately, in the absence of a regression analysis, the strength of the relationship between dentition and bacterial numbers could not be established.

Terpenning et al. (2001) prospectively evaluated medical and dental factors associated with AP among elderly veterans ($N = 358$). Among dentate patients, the number of decayed teeth significantly predicted the development of AP, with an odds ratio of 1.2. These findings confirm the earlier work by Langmore et al. (1998), however both the Terpenning et al. and Langmore et al. studies included only male participants, grossly limiting the generalizability of results. Unfortunately, although bacterial testing of dental plaque and saliva was undertaken, no comparisons were made between edentulous and dentate patients.

Denture-use has also been found to alter the oral microflora. Sumi, Miura, Sunakawa, Michiwaki and Sakagami (2002) documented the prevalence of potential respiratory pathogens from the denture plaque of dependent elderly (N = 50, age range = 60-89 years). Participants were dependent on caregivers for oral hygiene, had no evidence of active oral disease or diabetes and had not received antimicrobial therapy in the preceding 8 weeks. Dry dentures were swabbed, and the swabs were analysed for microbes via culture method. Aerobic bacteria were found to be present in 100 % of samples and potential respiratory pathogens were present in 46 %. The authors concluded that denture plaque is a specific site of colonisation of potential respiratory pathogens and control of this plaque is an essential step in the prevention of AP among dependent, denture-wearing elderly. However, in the absence of a control group of denture-wearing, independent elderly, it is impossible to say whether the results were a function of denture-use or dependence for oral care.

It is difficult to determine the true impact of dentition and denture-use on the oral microflora. This is partly because it is difficult to design a research study that controls for the many confounding variables. Some sources report that dentate adults appear to be at greater risk of respiratory infection (Mojon et al., 1997; Yoneyama et al., 2002), with number of teeth predictive of pneumonia (Langmore et al., 1998; Terpenning et al., 2001). However, other sources do not agree (Binkley et al., 2009; Russell et al., 1999). It is true that respiratory pathogens have been isolated from the mouths of edentulous (Binkley et al., 2009), denture-wearing (Sumi et al., 2002) and dentate subjects (Sumi et al., 2007), suggesting that the most important factor in the development of illness is not dentition, but oral health and colonisation of pathogenic bacteria.

The combined results from Sumi et al. (2002) and Sumi et al. (2007) complement Langmore et al.'s (1998) finding that dependence for oral care is a risk factor for pulmonary infection. These studies succeeded in identifying dependent, dentate elderly as a population who are at a significant risk for oral colonisation of respiratory pathogens (more so than their dentate counterparts). However, further examination of patient comorbidities/additional risk factors for bacterial colonisation is required in order to better identify patients at risk for AP.

5.1.3.3. Diabetes mellitus.

The presence of certain comorbidities also plays a role in determining the characteristics of an individual's microflora. Although many different morbidities predispose individuals to infection, diabetes mellitus is worthy of mention because of its high prevalence among the elderly (15 % in the over 75 years age group [Ministry of Health, 2015]) and specific links to periodontal infection in adults (Berkey & Scannapieco, 2013; Salvi, Lawrence, Offenbacher, & Beck, 1997). The likely mechanism for this is through changes to the individual's immunological defence functions, leading to reduced resistance to opportunistic, pathogenic bacteria (Mealey & Rose, 2008; Skerrett et al., 1989). This can have significant implications for respiratory health. Using strict criteria for the diagnosis of aspiration pneumonia, Terpenning et al. (2001) found that the presence of diabetes mellitus predicted aspiration pneumonia with an odds ratio of 3.5 among elderly, dentate subjects and with an odds ratio of 1.7 for both edentulous and dentate subjects.

5.1.3.4. Dependence level.

Another factor that contributes significantly to an individual's oral microbiota is their level of dependence. The prevalence of potential respiratory pathogens within elderly rest-home residents is reported to be 46-65 % (Sumi et al., 2007, 2002), with rest

home residents exhibiting significantly poorer oral hygiene and significantly more oral pathogens compared to control subjects (Russell et al., 1999). Given that these reports do not include analyses of anaerobic bacteria, the true prevalence of potential respiratory pathogens in this population may be even greater.

It is well documented that elderly people living in rest homes are more likely to have poor oral health (Awano et al., 2008; Ortega et al., 2014; Pietrokovski et al., 1995; Russell et al., 1999; Sumi et al., 2007). Neither Sumi et al. (Sumi et al., 2007, 2002) nor Russell et al. (1999) completed logistic analyses to determine the effect of institutionalisation on oral bacteria and it is likely that other risk factors such as chronic illness, multiple medications, dysphagia, caregiver training and resources play a major role in determining the oral microflora of rest home residents. It is also worth noting that the actual amount of oral bacteria may be vastly underestimated in the literature, as studies typically only involve subjects who are well enough to participate in examinations.

Changes to the oral microbiota do not only occur in residents of long-term care facilities. Observational studies of elderly hospitalised patients (Preston, Gosney, Noon, & Martin, 1999), patients with acute stroke (Zhu, McMillan, McGrath, Li, & Samaranayake, 2008) and patients admitted to a critical care unit (Sachdev et al., 2013) have all reported a high prevalence of Gram-negative bacteria. It is difficult for studies of this nature to include control participants. However, in the absence of controls it is difficult to determine the true influence of hospitalisation on the oral microflora.

Several studies have been completed which have included control patients, with similar findings. For example, Gram-negative bacteria are reported to be present in 34-42 % of oral swab/rinse samples of patients with acute stroke (Gosney, Millns,

Martin, & Field, 1997; Millns, Gosney, Jack, Martin, & Wright, 2003) – significantly less than healthy, elderly controls. Interestingly, long-term patients on rehabilitation wards (Millns et al., 2003) and psychiatric wards (Johanson, Pierce, & Sanford, 1969) also show very few of these bacteria suggesting the presence of Gram-negative bacteria cannot be entirely accounted for by hospitalisation and that acute illness and age likely play a role. Recovering the ability to independently manage oral hygiene routines may also explain this pattern of bacterial colonisation.

Among people who are physically well, there are no differences in the amount of Gram-negative bacteria despite differences in exposure to the hospital environment, from no-exposure (community-dwellers) to intermittent exposure (medical staff) to continuous exposure (patients on a psychiatric ward) (Johanson et al., 1969). This finding suggests that it is not the hospital environment that contributes to changes in oral microflora.

Studies using repeated-measures designs have provided more information about the effect of hospitalisation on oral bacteria. Zhu et al. (2008) quantified the changes in oral bacteria in elderly patients with acute stroke during their admission to hospital, at discharge from hospital and at six months. Oral carriage of Gram-negative bacteria peaked immediately following admission to hospital and progressively declined over six months. This was not associated with other variables such as age, stroke severity, use of dentures, difficulty tooth-brushing, etc. Sachdev et al. (2013) reported a similar result in patients admitted to a critical care unit. A significant increase in microbe counts from baseline to week one was documented, with bacteria associated with hospital-acquired pneumonia present in 26 % of patients. Combined results of these studies suggest that patients may be the most vulnerable to increased oral/pathogenic bacteria in the early stages of hospitalisation. Indeed, the risk may be under-estimated

by these studies, as sampling methods were limited to Gram-negative/anaerobic bacteria. Acutely-ill patients are more likely to have impaired pulmonary clearance (Johanson et al., 1969) and depend on others to provide oral hygiene. Importantly, these are all recognised as independent risk factors for pneumonia among elderly (Harkness, Bentley, & Roghmann, 1990; Langmore et al., 1998; Quagliarello et al., 2005; Terpenning et al., 2001). It has been recognised that improving the oral hygiene of dependent elderly is essential for the prevention of pneumonia in this population (Sumi et al., 2007).

5.1.3.5. Oral hygiene.

When tooth-brushing and oral hygiene routines are ceased, dental plaque will proliferate, taking only three or four days to reach maximum growth (Wilson, 2005). The microbial composition of the dental surfaces will continue to change and the proportion of Gram-negative species will increase (Wilson, 2005). This is due to the ability of bacteria to respond rapidly to changes in their environment by replicating quickly (Shiffer Nield-Gehrig & Willmann, 2007). Conditions that favour pathogenic bacteria may result in large numbers of pathogenic bacteria in the oral cavity within a relatively small timeframe.

It is also possible for bacteria to alter the host environment. Scannapieco and Mylotte (1996) proposed a model of respiratory pathogen colonisation in the oral cavity where pathogenic bacteria may adhere to and colonise the dental plaque in patients with poor oral hygiene. Poor oral hygiene is known to increase dental plaque load. The colonised bacteria produce increased amounts of hydrolytic enzymes in the saliva, which in turn modifies the proteins present on the mucosal surface, exposing these surfaces and promoting further colonisation.

A causal link has been established between poor oral hygiene and increased numbers of potential respiratory pathogens in oropharyngeal secretions (Loesche & Lopatin, 1998; Mojon & Bourbeau, 2003; Mojon et al., 1997; Scannapieco, 1999). Such pathogens possess several virulence features which make them more likely to lead to infection than other oral bacteria (Russell et al., 1999; Scannapieco, 1999).

Elderly people have been identified as particularly susceptible to changes in the oral microflora due to poor oral hygiene. A study of rest home residents reported that only one quarter of residents cleaned their own teeth (Carter et al., 2004). The other three-quarters relied on others for oral hygiene. This number may in fact be an underestimation, as only residents who were able to consent and considered well enough to be involved in this study were recruited.

The long-term effects of poor oral hygiene on the respiratory system have also been documented. Awano et al. (2008) first documented the relationship between periodontal disease and pneumonia-related mortality among the elderly in a large cohort of rest-home residents ($N = 697$). At the start of this four-year longitudinal study, oral examinations took place as well as demographic surveys and the sampling of the lingual surface for anaerobic bacteria and fungi. At the end of the study period, the death certificates of participants who had died were reviewed for the cause of death. Results revealed that having more than ten diseased teeth was an independent predictor of pneumonia-related mortality. There was a non-significant trend towards increased oral anaerobic bacteria (as measured using gas chromatography) and prevalence of *Candida* in participants who died from pneumonia. Using more direct measures of oral bacteria, such as polymerase chain reaction (PCR) analysis, may be required to understand this relationship more clearly.

As previously mentioned, Binkley, Haugh, Kitchens, Wallace and Sessler (2009) used molecular analysis to determine the relationship between oral hygiene, the prevalence of target bacteria and respiratory status in middle-aged adults ($N = 63$) dependent for oral hygiene. Monthly assessments included oral examinations, oral sampling and medical record reviews over a six-month period. PCR analysis was used to determine the presence of *S. pneumoniae,* MRSA*, Prevotella melaninogenica* and *Candida albicans.* Baseline analysis revealed a high prevalence (55 %) of potential respiratory pathogens and "fair" oral hygiene. There were twelve incidents of pneumonia over the study period (19 % of participants). Participants who had any of the target microbes in their baseline samples were more likely to experience respiratory infection and those with poor oral hygiene were more likely to develop pneumonia. The limited number of assays completed means that the type/prevalence of pathogenic oral bacteria may have been underestimated and unfortunately, as no formal measurements of the oral hygiene provided to participants were undertaken, it is difficult to make firm statements about the relationship between oral hygiene, bacteria and pneumonia.

Patients with stroke are at an increased risk of poor oral hygiene due to a sudden change in their ability to perform daily oral care routines such as a new hemiparesis, confusion or ataxia. Despite this, there are few published reports of how the oral microbiota changes in the days and weeks following an acute stroke. Given that morbidity and mortality from pneumonia is also particularly high in this population (Scannapieco & Mylotte, 1996), there is a critical need for research that clarifies the relationship between stroke, oral bacteria and pneumonia. However, it is important to note that poor oral hygiene and elevated oral pathogenic bacteria in isolation is not sufficient to cause aspiration pneumonia (Langmore et al., 1998;

Loesche & Lopatin, 1998). Respiratory pathogens must also be aspirated in such quantities or virulence that they overwhelm the respiratory system's mechanical and immunological defence systems, leading to respiratory infection (Marik, 2001). Therefore, future research should also focus on airway protection and how this may influence the risk of developing infection.

5.1.3.6. Stroke.

Changes to mastication, salivation, swallowing, the ability to self-feed and the ability perform oral hygiene routines are all common consequences of stroke which may have the potential to disturb the microbial equilibrium of the oral cavity and lead to colonisation by potential respiratory pathogens. For example, although stroke is associated with AP (Yamaya et al., 2001), few studies had examined the relationship between oral bacteria and AP in this population until recently.

In a prospective longitudinal study of 56 elderly Chinese patients with stroke, oral microbiological sampling revealed that oral yeast carriage was closely related to level of stroke-related functional disability (Zhu et al., 2008). Microbial sampling took place at three points in time: during acute hospitalisation, at discharge from hospital and at six months post-discharge. The average age of patients was 66 years, with approximately 75 % wearing dentures. The oral carriage of yeasts was found to be significantly increased during the acute stroke phase ($p < .05$), whereas coliform carriage was not. By six months, carriage of both yeasts and coliforms had significantly reduced compared to acute levels, indeed 92 % of patients who had evidence of oral coliforms at admission tested negative at subsequent sampling points. Seven coliform species were identified; interestingly, none were present across all three sampling periods. *Candida albicans* and *K. pneumoniae* were the most prevalent yeast and bacteria, respectively. Of particular interest, both microbes are known to be

associated with aspiration pneumonia, either directly (*K. pneumoniae*) or indirectly as a reflection of reduced host immunocompetence (*C. albicans* [Pace & McCullough, 2010; Zhu et al., 2008]). High oral yeast carriage was directly associated with stroke-related difficulty with oral care (i.e. tooth/denture brushing).

However, findings were tempered by several methodological constraints. First, the lack of a control group from which to draw comparisons regarding usual microflora environments and oral hygiene regimes contributes to weak overall validity. The authors attempted to make comparisons by citing results from other studies; however, given the difference in study populations (e.g. Western versus Asian) and methodologies, these were of limited use. Second, while evidence-based techniques were applied to oral microbiological sampling and identification, the authors failed to take precautions in their methodology when they administered an oral rinse to obtain bacteria samples. Specifically, there was an increased risk of participants (all of whom had diagnosed dysphagia) aspirating the rinsing liquid. The unfortunate implication is that this limits the extent to which this study can be replicated. Future studies using this technique should consider safer methods of outcome measurement. Such studies in this line of research could also include other stroke subtypes, e.g. brainstem, to increase generalizability of results, as well as indications of stroke severity, which may reveal those patients who are most at-risk for increased oral bacterial colonisation. Finally, the methods used in this study to culture the oral bacteria for identification were singularly suited to Gram-negative bacteria. The rationale for this was not stated, however the unfortunate implication is that the potential presence of Gram-positive bacteria and the implications of this have been overlooked.

Nevertheless, this study provided a very clear link between patients in the acute stages of stroke and the colonisation of pathogenic oral bacteria. This population is at increased risk for aspiration of oral intake and subsequent pneumonia. Steps to prevent the colonisation of pathogenic bacteria in the oral cavity may be one way to prevent AP in post-stroke patients.

5.1.4. Bacteria associated with acute stroke.

In the acute stages of stroke, patients may be severely unwell with reduced consciousness, reduced movement down one side of the body (hemiplegia/ hemiparesis), reduced salivary flow or reduced control of secretions and poor swallowing. These conditions may all contribute to alter the composition of oral bacteria, either by altering the environment of the oral cavity or by preventing usual oral hygiene routines from being carried out. For this reason, there may be specific bacteria/patterns of colonisation that are unique to patients with acute stroke.

Millns et al. (2003) obtained samples from the mouths of three groups of hospitalised patients: 1) patients with a first-time acute stroke, 2) patients from a stroke rehabilitation ward, 3) patients from a general (non-stroke) ward. A control group of healthy, elderly volunteers was also included. Samples from participants in groups 1 and 3 were collected using mouth swabs every three days for a total of eighteen days, whilst samples from participants in group 2 and control participants were collected using mouth-rinse samples every three days for the same period of time. Samples containing oral bacteria were cultured under aerobic conditions and analysed for the presence of any aerobic gram-negative bacteria colonies. The criterion for bacterial carriage was the presence of ten or more colonies on two or more consecutive samples. This was then compared to participants' clinical outcomes (i.e. mortality) and results of a bedside swallowing evaluation (completed for all

patient groups but not for control participants) by means of a correlation analysis. Carriage of Gram-negative aerobes was documented in 34 % of participants with acute stroke, 4 % of participants on the stroke rehabilitation ward, 8 % of acutely ill hospitalised patients and in none of the control participants. The predominating species were *Escherichia spp., Klebsiella spp., Enterobacter spp., Morganella spp.* and *Proteus spp.* Of note, 24 % of participants with acute stroke and carriage of Gram-negative aerobes also had dysphagia. This number rose to 36 % when only the deceased acute stroke patients were considered. These results suggest that patients with acute stroke and dysphagia are at increased risk for the carriage of pathogenic oral bacteria and that the presence of these bacteria is a poor prognostic indicator. It appears that neither acute illness nor hospitalisation alone can account for these findings, suggesting that the presence of stoke and dysphagia may be factors in the carriage of oral pathogens. Age may also play a role, contrary to the authors' interpretation. The median age of the healthy controls (71 years) differed by at least 13 years from the two groups with the highest rates of carriage (84 and 89 years). The authors also considered the relative lack of denture-wearing in the acute stroke group to be an argument against the theory that denture-wearing increases pathogenic bacterial carriage. The fact that the acute stroke group had one of the lowest rates of denture use may in fact suggest that the ability to remove and adequately clean dentures is a protective factor against pathogenic bacterial carriage.

The results of this study were limited by several confounding variables, including poor age-matching of participants and the use of two different bacterial sampling techniques. The authors stated that mouth swabs were used for patients who were considered at risk of aspiration, as the mouth-rinse technique may have increased this risk. However, it is unclear why oral swabs were not used for all

participants, as the use of two different sampling techniques introduces a confounding variable. Indeed, the two groups who were sampled using oral swabs tested the highest for pathogenic bacteria. Finally, by only testing samples for Gram-negative aerobes that could grow under lab conditions, validity and sensitivity are diminished.

The results presented by Millns et al. (2003) suggested that acute stroke patients behave differently from other people and other hospitalised patients. However, the microbiological characteristics of patients with stroke who develop aspiration pneumonia remained unknown. Several research groups since have contributed to the literature on this topic. A summary of these findings, with particular attention to investigations of non-ventilated, hospital-acquired pneumonia, is presented in Table 4.

5.1.4.1. Characteristics of bacterial pathogens associated with post-stroke aspiration pneumonia.

As previously mentioned, the presence of oral pathogens is necessary, but not sufficient, for respiratory disease to develop (Langmore et al., 1998; Pace & McCullough, 2010; Socransky & Haffajee, 1992). Socransky and Haffajee described seven criteria that must be met in order for a pathogenic microbe to cause disease: 1) the pathogen must be virulent (i.e. infectious) and clonal (i.e. a single cell is able to produce many cells), 2) it must possess the genetic factors to initiate disease, 3) it must be present in numbers that exceed the host's threshold, 4) it must be in the right environment, 5) the environment must select for the expression of the pathogen's virulent properties 6) other bacteria must foster/not inhibit the pathogen, and 7) the host must be immunocompromised.

Sources differ as to which bacteria fit the above criteria and can therefore be considered as precursors to aspiration pneumonia. Traditionally, it was thought that

Table 4.

Summary of Literature Characterising the Oral Microbiota of Patients with Aspiration Pneumonia.

Reference	Population	Pneumonia sub-type	Outcome measurement method	Identified bacteria	Comments
Kollef et al. (2005)	Hospitalised patients	Non-ventilated, hospital-acquired pneumonia	Respiratory culture samples	*Acinetobacter* sp. *Enterobacter* sp.* *Escherichia* sp.* *Haemophilus* sp. *Klebsiella* sp.* *Pseudomonas* sp.* *S. aureus** *S. pneumoniae**	
Zhu et al. (2008)	Hospitalised patients with stroke	Not reported	Oral samples measured via culture methods	*Enterobacter gergoviae* *Chryseomonas luteola* *K. pneumoniae**	

El-Solh et al. (2003)	Elderly, rest-home residents	Aspiration pneumonia	Samples obtained within four hours of admission to hospital using protected bronchoalveolar lavage, cultured under aerobic and anaerobic conditions	*Bacteroides* spp. *E. clocae** *E. coli** *Fusobacterium* spp. *H. influenza* *K. pneumoniae** *Peptostreptococcus* spp. *Proteus mirabilis* *Serratia* spp. *S. aureus** *S. pneumoniae**	
Ewan et al. (2015)	Elderly patients with lower limb fracture	Hospital-acquired pneumonia	Oral swabs analysed using molecular testing methods	*E. coli** *P. aeruginosa** *S. aureus**	Some patients received antibiotics and/or clorhexidine prior to testing

Marik & Careau (1999)	Hospitalised patients, including stroke	Aspiration pneumonia	Samples obtained via protected specimen brush sampling and bronchoalveolar lavage, cultured under aerobic and anaerobic conditions	*E. clocae** *E. coli** *Flavobacterium* spp *H. influenza* *K. pneumoniae** *Serratia* spp. *S. aureus** *S. pneumoniae**	48 % of patients received antibiotics prior to sampling
Sachdev et al. (2013)	Patients admitted to an intensive care unit	Not reported	Dental plaque samples cultured under aerobic and anaerobic conditions	*E. clocae** *K. pneumoniae** *P. mirabilis* *P. aeruginosa** *S. aureus**	Study aim was to document presence/changes in pathogens associated with pneumonia. Pneumonia itself was not a measured outcome.

Bousbia et al. (2012)	Patients admitted to an intensive care unit	Aspiration pneumonia	Samples obtained via bronchoalveolar lavage and analysed using molecular testing methods	*E. coli** *H. influenza* *K. pneumoniae** *Peptostreptococcus* spp. *P. aeruginosa** *S. aureus** *S. mitis* *S. pneumoniae**	
Wei et al. (2013)	Patients admitted to an intensive care unit	Aspiration pneumonia	Samples obtained via bronchoalveolar lavage and analysed using culture testing methods.	*Acinetobacter baumannii* *K. pneumoniae** *P. aeruginosa** *S. aureus**	Anaerobic bacteriology not completed.

Terpenning et al. (2001)	Elderly, rest-home residents	Aspiration pneumonia	Saliva, mucosa and plaque samples analysed under aerobic and anaerobic conditions	*Porphyromonus gingivalis* *S. aureus** *Streptococcus sobrinus* *Bacteroides* spp. *Fusobacterium* spp. *K. pneumoniae** *Peptostreptococcus* spp. *S. pneumoniae**
Lorber & Swenson (1974)	Hospitalised patients	Hospital- or community-acquired pneumonia	Samples obtained via transtracheal aspiration or thoracentesis and cultured under anaerobic and aerobic conditions	*E. coli** *P. aeruginosa** *S. aureus**
		Hospital-acquired pneumonia		

Hassan et al. (2006)	Patients with acute stroke	Community-acquired pneumonia	Tracheal samples were analysed using culture-based methods	*P. aeruginosa**	Excluded patients who developed fever prior to diagnosis of pneumonia. Tracheal cultures only performed in patients who were suspected to have pneumonia.
				*S. aureus**	
				*E. coli**	
				*S. pneumoniae**	
				Acinetobacter sp.	
				Enterococci coli	
		Hospital-acquired pneumonia		*P. aeruginosa**	
				*S. aureus**	
				*E. coli**	
				*S. pneumoniae**	
				Acinetobacter sp.	
				Enterococci coli	
				*K. pneumoniae**	

Note. HAP = Hospital-acquired pneumonia. * = Bacterial species that have been implicated in the development of pneumonia in more than five published studies.

anaerobic Gram-negative bacteria were responsible for causing aspiration pneumonia among elderly people, as was reported in three landmark studies from the 1970s (Bartlett et al., 1974; Cesar et al., 1975; Lorber & Swenson, 1974). Sources have continued to report this (e.g. Awano et al., 2008; Pace & McCullough, 2010; Scannapieco, 2006), despite the fact that early studies were grossly limited by the technology available at the time and plagued by other methodological constraints. For example, in each of the early studies the microbiological samples used for analysis were acquired late in the course of the pneumonia, by which time other complications had developed. Furthermore, many of the participants were reported as having chronic alcoholism, a condition that is not necessarily reflected in the typical patient with acute aspiration pneumonia and there was often no differentiation between community-acquired and hospital-acquired pneumonia. Finally, because bacteria often require specific transport and culture conditions, it is unlikely that precise data regarding bacterial colonisation were obtained.

More recent data, including that cited in Table 4, suggest that the aetiology of aspiration pneumonia may actually be polymicrobial, involving both Gram-negative and Gram-positive bacteria. Despite considerable work in this area, it is difficult to synthesize findings due to differing criteria for aspiration pneumonia, differences in bacterial sampling and analysis methods and lack of distinction between hospital-acquired pneumonia, aspiration pneumonia and aspiration pneumonitis. Studies are also limited by retrospective designs (e.g. Hassan et al., 2006; Kollef et al., 2005), inclusion/exclusion biases (Hassan et al., 2006) and narrow bacteriology (Wei et al., 2013; Zhu et al., 2008). This makes it difficult to determine the precise bacterial aetiology of aspiration pneumonia.

It is generally accepted that molecular-based bacterial analysis methods, such as those described by Bousbia et al. (2012) and Ewan, Sails, Walls, Rushton and Newton (2015) are likely to give a more accurate picture of the microbiological environment. Bousbia et al. (2012) attempted to characterise the microbiota of patients who were admitted with or developed pneumonia in intensive care units compared to controls without pneumonia. The prevalence of several bacteria that are directly or indirectly related to ventilator-associated pneumonia (VAP; $n = 106$), community-acquired pneumonia (CAP; $n = 32$), non-ventilator ICU pneumonia (NVP; $n = 22$) and AP ($n = 25$) was examined over a three-year period. Control data were obtained via bacterial clone libraries and included 'immunocompromised' controls ($n = 25$). Bacterial samples from ICU patients were extracted via bronchoalveolar lavage, and analysed using an extensive variety of traditional and moderen laboratory techniques, including the amplification and quantification of DNA from specific pathogens as well as culture-based testing. Patients with AP had the highest prevalance of *S. mitis* and *S. pneumoniae* compared to patients with VAP, CAP, NVP, and controls. *S. mitis* with origins in oral flora and dental plaque was detected in 12 % of patients with AP, compared to 6 % of patients with CAP, 12 % of patients with VAP, 9 % of patients with NVP and 4 % of controls. *S. pneumoniae* with origins from nasopharyngeal mucosa was detected in 24 % of the AP cohort, versus 6 % of patients with CAP, 6 % of patients with VAP, 5 % of patients with NVP and 0 % of controls. By associating *S. mitis* and *S. pneumoniae* – two Gram-positive, facultative bacteria – with AP, Bousbia et al.'s (2012) findings contrast with the traditional view that AP is caused almost exclusively by Gram-negative bacteria (Awano et al., 2008; Pace & McCullough, 2010; Scannapieco, 2006). In fact, Bousbia

et al. (2012) reported that Gram-negative bacteria were only detected in very low levels in the AP group.

One possibility for these contrasting findings could be related to methodology, specifically sampling and diagnostic techniques. Previous work has focussed on standardised routine culture tests (e.g. blood culture) and culture of lower respiratory tract specimens. However, these diagnostic tests have been described as having "suboptimal diagnostic sensitivity" for diagnosing pneumonia (Murdoch et al., 2009). For example, blood cultures are positive in <10 % of patients with pneumonia (Murdoch et al.). Bousbia et al. (2012) accounted for this by combining standard bacterial testing with molecular testing techniques (i.e. qPCR). Some advantages of molecular testing include the ability to detect potentially all respiratory pathogens even when present in very low levels and reduced liklihood of results being affected by antibiotic administration compared to culture-based methods (Murdoch et al.). Indeed, by including molecular testing, Bousbia et al. identified 73 bacterial species that had not previously been reported in the literature. Arguably, their extensive arsenal of diagnostic tests increased the accuracy of their results, lending support to the hypothesis that *S. mitis* and *S. pneumoniae* may be the main culprits in AP.

More recently, Ewan et al. (2015) investigated the carriage of pathogenic oral bacteria at several time points in elderly hospitalised patients ($N = 90$). In this study, molecular-based bacterial analysis (i.e. qPCR) was used to detect and quantify seven potential respiratory pathogens in tongue and throat swabs obtained at five time points over a two-week period. Importantly, the participants were all considered to be healthy other than having a lower limb fracture. The seven target bacteria were *S. aureus,* MRSA*, E. coli, P. aeruginosa, S. pneumoniae, H. influenza* and *Acinetobacter* spp. Patients were followed for a period of 90 days and the incidence of

HAP was recorded. Results revealed that ten of the study participants developed HAP defined by the somewhat subjective criterion of 'clinician-initiated antibiotics'. If the more rigorous criteria for lower respiratory tract infection (LRTI) described by the American Thoracic Society/British Society for Antimicrobial Chemotherapy guidelines were considered, seven out of 90 participants developed HAP/LRTI. Four bacterial species were significantly associated with the development of HAP: *E. coli, S. aureus,* MRSA and *P. aeruginosa.* If two or more of these bacteria were present, the odds of developing HAP increased by 9.4. In 90 % of participants, colonising (i.e. present on two or more occasions) bacteria were present within 72 hours of admission to hospital. The association between HAP and carriage of *E. coli/S. aureus/*MRSA*/P. aeruginosa* at day five or fourteen of admission was statistically significant.

The study by Ewan et al. (2015) was limited by incomplete participant recruitment and data collection resulting in low statistical power, as well as an inclusion bias towards "well" patients, making generalisation to other groups of patients, such as patients with acute stroke, difficult. However, it is one of the only studies to date to systematically explore the association between pathogenic oral bacteria and HAP over several time points using robust bacterial analysis techniques and in doing so has highlighted several key pathogens that may be worth targeting in future research.

To date, the particular species implicated in the development of AP in patients with acute stroke and dysphagia remains undocumented. The relationship between pathogenic bacteria and AP over time in this population also remains unknown. Based on the existing literature, six bacteria in particular may play a role in the pathogenesis of AP in the acute stroke population: *S. mitis, S. pneumoniae, S. aureus, K.*

pnuemoniae, E. coli and *P. aeruginosa.* Further investigation into this area is warranted.

5.1.5. The relationship between oral care and aspiration pneumonia.

In a large-scale, randomised controlled trial (RCT), Yoneyama and colleagues (2002) demonstrated that implementing regular tooth brushing by nursing staff three times per day combined with professional dental cleaning once per week amongst elderly rest home residents ($N = 417$) reduced the rate of pneumonia by 8 %, as well as significantly reducing the number of febrile days and death from pneumonia. Participants in this study had a range of aetiologies, including stroke. These findings suggest that not only does improving oral health decrease the risk of contracting pneumonia, but that oral care was more effective at preventing death from pneumonia than was medical intervention (e.g. antibiotics).

Interestingly, there appear to be secondary benefits of improving oral hygiene. Yoshino, Ebihara, Ebihara, Fuji, and Sasaki (2001) documented decreased swallowing latency in a group of rest home patients with cerebrovascular disease ($n = 20$) who underwent a daily, thorough oral care regime provided by their caregivers for 30 days. The same effect was not observed among control patients ($n = 20$) who performed their own oral care. The authors hypothesised that increased stimulation of the sensory nerves in the oral cavity triggered the release of increased amounts of neuropeptides, which subsequently affected swallowing. Indeed, when tested, participants in the oral care group had larger amounts of serum substance P, a neurotransmitter known to play a role in swallowing elicitation, than participants who performed their own oral care.

The same research group went on to investigate the effect of intensive oral care on cough sensitivity among rest home patients (Watando et al., 2004). Patients

were predominantly elderly, with a range of aetiologies including stroke, dementia, mild cognitive impairment, and diabetes mellitus. Some patients required assistance with eating, while others were independent, but all patients received nutrition orally. Patients were randomly assigned to one of two groups: a treatment group who received intensive oral care (n = 30) or a control group who performed their own (occasional) oral cares (n = 30). The treatment group received mechanical tooth brushing from nursing staff following every meal, in addition to professional dental cleaning from either a dentist or dental hygienist once per week. After three days, ten days, and 30 days, cough reflex sensitivity was measured by a citric acid cough reflex test (CRT). While both groups were comparable for cough reflex sensitivity at baseline, results identified a significant increase in cough reflex sensitivity among the treatment group at 30 days, regardless of whether patients were edentulous or not. No such change was observed in the control group. The authors concluded that intensive oral care was effective at reducing aspiration pneumonia in elderly rest home patients via increased cough reflex sensitivity.

All three of these randomized controlled trials provide a high quality of evidence due to their meticulous control of confounding variables and avoidance of bias. A combination of prospective design, group randomisation, and presence of control group gives weight to the idea that, in each study, intensive oral care accounted for the observed treatment effects. On the other hand, a lack of reported blinding during outcome measurement decreases validity.

A point of difference in the Watando et al. (2004) study is that participants in their treatment group not only received oral cares three times per day from their caregivers, but also received professional dental care once per week from either a dentist or professional oral hygienist. Thus it remains uncertain whether the observed

treatment effects were as a result of the daily oral cares, weekly professional cleaning, or a combination of both.

In elderly rest home populations, intensive oral hygiene may reduce pneumonia rates may by improving the swallowing reflex (Yoshino et al., 2001) and increasing cough reflex sensitivity (Watando et al., 2004). Future studies exploring the effects of daily oral care versus weekly professional cleaning on health outcomes may be crucial in determining recommendations for future care in this population.

5.2. Summary

In most people, the oral microflora exist in a state of equilibrium. A variety of individual factors such as dentition, diet, self-care, medications and drug-use all contribute to an individual's oral bacterial profile. The human oral microbiome has been extensively studied and recent advances in technology have allowed sampling to take place at the molecular level, allowing a more complete picture of both the healthy and diseased oral microbiome to emerge.

Over several decades of research, the link between increased oral bacteria/oral colonisation by pathogenic bacteria and respiratory illness has become clear. What is less clear is the degree to which individual risk factors play a role in the pathogenesis of oral – and subsequent respiratory – disease, specifically, aspiration pneumonia. Increased age (Percival et al., 1991; Tachibana et al., 2006), increased level of independence (Mojon et al., 1997; Russell et al., 1999; Sumi et al., 2007, 2002; Yoneyama et al., 2002), diabetes mellitus (Terpenning et al., 2001), poor oral hygiene (Loesche & Lopatin, 1998; Mojon & Bourbeau, 2003; Mojon et al., 1997; Scannapieco, 1999) and acute hospitalisation (Sachdev et al., 2013; Zhu et al., 2008) have all been implicated as risk factors for respiratory infection. Because many of these variables are related, it may be impossible to determine the exact contribution of

each to an individual's risk of aspiration pneumonia. What is striking is that many of these risk factors are also present in patients with acute stroke. Despite this, there are few published reports of how the oral microbiota changes in the days and weeks following an acute stroke. Given that morbidity and mortality from pneumonia is also particularly high in this population (Scannapieco & Mylotte, 1996) this represents a critical gap in the evidence base surrounding pneumonia prevention. The relationship between stroke, oral bacteria and pneumonia requires urgent clarification.

To date, the particular species implicated in the development of AP in patients with acute stroke and dysphagia also remains undocumented. Based on the existing literature, six bacteria in particular may play a role in the pathogenesis of AP in the acute stroke population: *S. mitis, S. pneumoniae, S. aureus, K. pnuemoniae, E. coli* and *P. aeruginosa.* Further investigation into this area is warranted.

The presence of oral bacteria is also strongly aligned with adverse pulmonary consequences. Protocols that reduce oral bacterial loading and colonisation of pathogenic microorganisms reduce the risk of pneumonia (Simmons-Trau et al., 2004; Bousbia et al., 2012; Yoneyama et al., 2002; Yoshino et al., 2001; Watando et al., 2002). This reduction in pneumonia can easily be attributed to a reduction in oral pathogens. However, Watando et al. (2002) identified another direct effect of oral hygiene: improved cough reflex sensitivity. This suggests that intensive oral care can increase airway protective mechanisms. However, it remains unknown whether improved cough reflex sensitivity is due to decreased oral bacterial load, increased mechanical stimulation of the oral cavity or a combination of both. Clarification through further investigation is required if we are to provide appropriate interventions for patients at risk of AP. Furthermore, despite a known increase in oral carriage of yeasts in acute stroke, there are no specific studies documenting the relationship

between oral bacteria, cough sensitivity and pneumonia in the acute stroke population. Given that this population is at substantial risk of both aspiration and consequent pneumonia, this represents a significant gap in the knowledge base supporting clinical care.

Chapter 6. Methods of Characterizing the Oral Microbiota

6.1. Methods of Obtaining Oral Bacterial Samples

There are several widely-used methods for obtaining oral bacterial samples. One commonly-used method in clinical research is protected specimen brush sampling, whereby a small brush is inserted into the lungs via a bronchoscope to collect a sample, then retracted into a plastic tube to avoid contamination from the throat and oral cavity. This method, combined with quantitative analysis, is considered to be the gold standard in diagnosing nosocomial pneumonia (Mertens et al., 1998). Another widely-reported technique is bronchoalveolar lavage. In this technique, a bronchoscope is passed through the nose or mouth, into the lungs. A small amount of liquid is squirted into the lungs and then re-collected for analysis. The disadvantages of both of these methods are that they require a specialist with expertise in the technique and they are highly invasive. Sputum sampling is a routinely-used technique to diagnose nosocomial pneumonia and invasive respiratory testing is rare unless the patient is mechanically-ventilated. The disadvantage of sputum sampling is that samples contain a mixture of bacteria from the upper and lower respiratory tracts as well as the oral cavity. Many patients also find it difficult to produce a sputum sample.

Saliva sampling is another option when attempting to measure oral bacteria. Secreted saliva contains few, if any, bacteria, however it gains up to 10^9 microbes per millimetre as it washes over the tongue, mucous membranes, gingival and periodontal tissues (Gibbons & van Houte, 1975). The concentrations of bacteria found in saliva fluctuate throughout the day, with the highest concentrations present during mastication, and upon waking (Gibbons & van Houte). Salivary testing is considered

to be a practical and reliable means of measuring exposure to risk factors for many systemic illnesses (Lawrence, 2002). Considering that AP is caused directly by aspirated oral bacteria, it seems logical that a patient with AP would have the same organisms present in the oral cavity as they would at the site of infection (i.e. the lungs). Bacterial markers in the saliva may even be present before the infection presents. Salivary testing typically involves the use of a cotton bud or similar device inserted into the mouth for a short period of time before removing the device and analysing the collected saliva. This may be an ideal alternative to other more invasive methods of bacterial sampling.

6.2. Methods of Analysing Oral Bacteria

6.2.1. Chemotaxonomic methods.

Chemotaxonomic methods classify bacteria based on chemical features such as the cell wall composition, lipids and proteins. This method may be particularly useful for the identification and classification of new bacterial species. There are several methods of chemotaxonomy, mainly involving variations of spectroscopy, chromatography and gel electrophoresis.

6.2.2. Phenotypic methods.

Analysis of bacteria in health research has traditionally relied on analysis of samples cultured under laboratory conditions. Bacteria are identified under microscopy based on their physical characteristics (morphology) and physiology. Culture-based techniques, however, present several limitations. Importantly, not all bacteria can be grown in laboratory conditions. Sample yields may be low, due to stress on the bacteria, fastidiousness and failure to thrive or slow growth (MacNeil, Kauri, & Robertson, 1995). Highly-related bacteria species with very similar phenotypes are not distinguishable. Successful growth often relies on timely delivery of samples to

the laboratory to ensure the detection of viable bacteria. Even when optimal growing conditions are met, growth under laboratory conditions may not accurately represent *in vivo* growth. Culture-based testing is biased towards bacteria which are easily and/or rapidly grown or samples that contain high concentrations of bacteria (MacNeil et al., 1995). Finally, greater than 99 % of bacteria found in any environment are not cultivable using standard culture techniques (Hugenholtz, 2002). Such limitations have led to the development of more sophisticated analysis techniques, namely, molecular testing.

6.2.3. Molecular methods.

The development of nucleic acid techniques in the 1980s revolutionised the field of microbiology as previously unculturable organisms could be analysed with relative efficiency and little cost. The polymerase chain reaction (PCR) is one of the most widely used technologies in molecular biochemistry (Fakruddin, Chowdhury, & Hossain, 2012) and will be the focus of this section.

6.2.3.1. Polymerase chain reaction (PCR).

PCR, also known as end-point PCR, can be used to amplify a specific piece of the DNA strand, known as the DNA target, in order to generate millions of copies of a particular DNA sequence. As the PCR progresses, the new DNA is itself used as a template for replication, resulting in exponential amplification of the target. This feature means that PCR has far higher sensitivity compared to traditional culture methods, whilst retaining high specificity (Tang, Procop, & Persing, 1997). Other benefits include improved efficiency of testing and the ability to identify dead or dormant microbes. The PCR is a well-established method and a summary of the reaction follows.

6.2.3.1.1. PCR procedures.

A thermal cycler alternately heats and cools the PCR sample according to a defined series of temperature steps. PCR typically consists of 20-40 repeated temperature changes (cycles). The entire process generally takes about one hour and can be summarized into six steps (Table 5).

6.2.3.1.2. Advantages and disadvantages of PCR

The main advantage of PCR over other methods of bacterial analysis is that bacteria which are unable to be cultured can be detected at the molecular level, reducing the risk of false-negatives. Comparisons of culture methods and molecular methods such as PCR suggest higher bacterial detection rates using PCR (Johansson, Kalin, Tiveljung-Lindell, Giske, & Hedlund, 2010; Saukkoriipi, Leskela, Herva, & Leinonen, 2004; Templeton et al., 2005) even when antibiotics have been taken (Strålin, To, Kaltoft, & Olce, 2006).

One of the disadvantages of PCR is that the DNA targets must be specified *a priori.* This is problematic because the user may not be aware of all of the organisms present in a sample, inadvertently excluding organisms of interest. When designing the primers, extreme care must be taken to use accurate sequence data. DNA sequences are publically available through databases such as GenBank, however these data must be manually validated to ensure accurate and viable PCR products. The end product will represent all amplified DNA from the sample, not just the target DNA, therefore, DNA sequencing of the product must be undertaken *post hoc* to be sure that the product is indeed the target DNA and not a similarly-sized DNA product.

Because the prevalence of oral bacteria is so high (100 million bacterial cells per 1 mL of saliva [Curtis et al., 2011]), it is essential that molecular analysis is highly specific to avoid false positive results. There are a number of specific molecular

Table 5.

The PCR process.

Stage	Process	Result
1. Denaturation	The reaction mixture is heated to 94-98 °C for 20-30 seconds.	DNA melting/disruption of the hydrogen bonds between complementary bases, leading to physical separation of the DNA double helix into single-stranded DNA molecules.
2. Annealing	The reaction mixture is cooled to 50-65 °C for 20-40 seconds.	Polymerase binds the primers to the DNA template and a new double-stranded DNA molecule begins to form.
3. Extension/elongation	The DNA polymerase synthesizes a new DNA strand that is complementary to the DNA template by adding dNTPs. Stages 1-3 are repeated 40 times.	The amount of DNA target doubles i.e. the DNA target is amplified exponentially.
4. Final elongation	The reaction mixture is held at 70-74 °C for 5-15 minutes.	Remaining single-stranded DNA are extended.

5. Final hold	The reaction mixture is cooled to 4-15 °C.	Storage of the product until analysis.
6. Analysis	Size analysis is performed using gel electrophoresis and sequencing	Results of PCR interpreted.

analyses that are suitable for analysing oral bacteria however, for research purposes, qPCR may be the most appropriate as it is widely available in laboratories, cost-effective and provides reliable, quantifiable, highly sensitive and rapid information about target DNA within a sample.

6.2.3.2. Quantitative PCR (qPCR)

qPCR, also known as real time PCR, combines PCR amplification and detection into a single process. qPCR follows the principles of PCR; it differs in that the amplified DNA is detected in 'real time' as the PCR progresses, negating the need for post-PCR size analysis. qPCR is preferable to other PCR formats for several reasons. Unlike size analysis, which distinguishes the weight of molecules with limited specificity, qPCR characterises molecules by their sequence and therefore is a more reliable and useful tool (Saunders, 2009). Similarly, unlike other PCR formats that rely on post-amplification manipulation and analysis, qPCR provides a quantitative outcome measure of the concentration of the target sequence in the sample in rapid time. Other advantages include the ability to constantly monitor the reaction, low contamination risk due to sealed reaction tubes, increased sensitivity and reproducibility, and software-driven operation (Logan & Edwards, 2009).

During qPCR, fluorescent dyes are used to label and detect PCR products during thermal cycling. qPCR instrumentation measures the accumulation of fluorescent signal during each cycle. The cycle in which fluorescence is detected is called the quantitation cycle (Cq). The Cq is the basic result of qPCR. Low Cq values correspond to high initial copies of the target. Each target molecule is copied once during each thermal cycle and data are recorded throughout.

There are two main strategies to fluorescently label the qPCR products: 1) target-specific DNA probes made up of oligonucleotides that produce a signal only

when the target DNA sequence is amplified (probe hybridisation; e.g. TaqMan® fluorogenic probes), 2) non-specific fluorescent dyes that bind to double-stranded DNA and emit fluorescence when bound (e.g. SYBR® Green I dye).

6.2.3.2.1. Detection of bacteria by probe hybridisation versus non-specific fluorescent dye.

Both methods of qPCR have advantages and disadvantages. The main advantages of probe hybridisation methods such as TaqMan® are high sensitivity and specificity, as fluorescence will only be generated when there is a specific reaction between the probe and target. This method is generally less susceptible to providing false-positive results (Saunders, 2009). TaqMan® is considered to be more sensitive than other methods for detecting low numbers (fewer than 10 copies) of target DNA (Bookout & Mangelsdorf, 2003). However, this may be irrelevant, as most qPCR instruments have limited sensitivity below 10 copies of target DNA. The other main advantage of TaqMan® is that up to two different sequences can be amplified and detected in one reaction tube (duplex). However, if more than two sequences are of interest, the synthesis of several different probes is required, and TaqMan® chemistry is costly to run.

The other method of qPCR is SYBR® dye chemistry. This method does not require probes, significantly reducing analysis costs. In general, the SYBR® dye and TaqMan® methods are considered to have comparable sensitivity (Schmittgen & Livak, 2008). However, because SYBR® dye binds to all DNA strands, it will bind to the target strands as well as any non-target strands. This increases the likelihood of generating false positive results. This can be managed by carefully designing primers that will not amplify non-target DNA strands and by performing melt curve analysis to check outcomes.

6.3. Summary

Despite the known links between oral bacteria and pneumonia, to date, there are no studies describing the relationship between oral bacteria and AP in patients with acute stroke. Considering that this population are likely to experience an acute, detrimental change to their oral hygiene routines during hospitalisation, this issue requires urgent attention. If changes in oral bacteria could be characterised during the acute stroke phase, this information could be used to inform oral hygiene protocols and potentially reduce the risk of AP.

A link between oral bacteria and cough reflex sensitivity in elderly rest-home residents has also been suggested (Watando et al., 2004). The mechanism behind this relationship remains unknown, but could be of great benefit to patients with acute stroke who are at risk of decreased cough reflex sensitivity and aspiration.

Salivary testing may be an ideal alternative to other invasive methods of bacterial sampling as it is fast, comfortable, relatively non-invasive and does not require expertise to perform. When attempting to measure bacteria associated with AP, testing of oral bacteria is relevant as the oral cavity is the presumed source of respiratory pathogens.

With recent advances in technology, molecular methods of bacteriology are often favoured over more traditional, culture-based methods as they provide enhanced sensitivity and overcome the issue of culturing live organisms. Within molecular-based biochemistry, both probe hybridisation and non-specific fluorescent dye methods have advantages and disadvantages. For exploratory science, non-specific fluorescent dye methods may be preferred as their relatively cost-effectiveness allows a greater number of targets to be analysed.

6.4. Summary of Evidence

Dysphagia, when accompanied by pathogenic oral bacteria, aspiration and poor pulmonary clearance, may substantially increase the risk of pneumonia in patients with acute stroke. Despite a documented increase in the oral carriage of pathogenic bacteria in this population compared to other patient cohorts (Millns et al., 2003), no studies have documented changes in oral bacteria in the days and weeks following an acute stroke. Given that morbidity and mortality from pneumonia is also particularly high during this period (Scannapieco & Mylotte, 1996), this represents a critical gap in the evidence base surrounding pneumonia prevention. Based on the existing literature, six bacteria in particular may play a role in the pathogenesis of AP in the acute stroke population: *S. mitis, S. pneumoniae, S. aureus, K. pnuemoniae, E. coli* and *P. aeruginosa.* The relationship between these bacteria, stroke, and pneumonia requires urgent clarification.

A link between oral bacteria and cough reflex sensitivity in the elderly has also been suggested (Watando et al., 2004), although the mechanism for this remains unknown. If the relationship between cough sensitivity and oral bacteria were clarified, this could inform interventions specifically targeting those at risk of pneumonia due to decreased cough reflex sensitivity.

Impairment of the cough reflex is a potential side-effect of stroke which can lead to silent aspiration. CRT is a sensitive and specific screen of silent aspiration (Imoto et al., 2011; Miles, McFarlane, Allen, & Huckabee, 2013; Sato et al., 2012; Wakasugi et al., 2008, 2014). However, a recent study investigating the usefulness of CRT in a clinical population found no effect on pneumonia or mortality rates (Miles et al., 2013a). There is evidence to support the use of standardised management protocols in dysphagia management (Addington, Stephens, & Gilliland, 1999;

Gandolfi et al., 2014; Ickenstein et al., 2010; Leder et al., 2012; Odderson et al., 1995), leading to the question of whether the incorporation of CRT into a clinical protocol may be effective in reducing AP.

CRT is increasingly utilised in research and clinical practice, with wide variations in methodology. Time of testing is considered to be a major confounding variable in CRT that is controlled for by testing at the same time of day. However, this may not be feasible in acute clinical settings. A pattern of diurnal variation in cough reflex sensitivity has only been described using the vital-capacity method (Pounsford & Saunders, 1985) and it remains unclear whether the same effect is present when the commonly used tidal breathing method of CRT is used, or when oral bacteria are controlled for. Clarification of this issue is needed to inform clinical and research practices around CRT.

Chapter 7. Objectives and Hypotheses

7.1. Study I: Diurnal Variation in Cough Reflex Testing

7.1.2. Research question.

Cough reflex testing is increasingly utilised in dysphagia research and clinical practice as a means of assessing reflexive cough sensitivity and silent aspiration risk. In 1985, Pounsford and Saunders described a strong pattern of diurnal variation in cough responsiveness to citric acid presented via a vital-capacity/mouthpiece method among healthy participants. Specifically, cough reflex thresholds were significantly lower when tested in the morning versus the afternoon. Since this study, several research groups have identified strong links between oral bacteria and pulmonary health (Bousbia et al., 2012; Simmons-Trau, Cenek, Counterman, Hockenbury, & Litwiller, 2004; Watando et al., 2004; Yoneyama et al., 2002; Yoshino et al., 2001). Watando et al. reported improved cough reflex sensitivity levels following intensive oral hygiene, suggesting a link between oral bacteria and the cough reflex. However, Pounsford and Saunders did not control oral bacteria levels. It is possible that oral bacteria represent a confounding variable. It is unknown whether diurnal variation in cough sensitivity exists when alternative cough reflex testing methods are used – such as the tidal-breathing method that is commonly used in clinical assessment – and oral bacteria are controlled.

7.1.3. Hypotheses.

1) There will be no significant difference in participants' cough reflex thresholds when measured in the morning compared to the afternoon.

7.1.4. Rationale.

To date, only a single study has described a pattern of diurnal variation in cough reflex sensitivity. If this pattern truly exists, it would represent a major confounding

variable in research and clinical practise. Further investigation into this aspect of cough reflex testing is warranted.

7.1.5. Significance.

Future research design as well as current clinical practice will benefit from answering the question, 'is time of testing a confounding variable in cough reflex testing using the tidal breathing/facemask method?'. Results from this study will also contribute to the body of evidence regarding the repeatability of the CRT.

7.1.6. Proposed study.

The aim of this study is to characterise patterns of cough reflex sensitivity in healthy adults throughout the day using cough testing methods currently used in clinical practice. Fifty-three participants (19 – 37 years) will undergo cough reflex testing on two occasions: once in the morning (between 9am – midday) and once in the afternoon (between 2 – 5pm). The order of testing will be counter-balanced. Within each assessment, participants will inhale successively higher citric acid concentrations via a facemask, with saline solution randomly interspersed to control for a placebo response. The lowest concentration that elicits a reflexive cough response will be recorded. Oral bacteria levels will be controlled for by participants' brushing their teeth immediately prior to testing.

7.2. Study II: Evaluation of a Dysphagia in Stroke Protocol in Patients with Acute Stroke

7.2.1. Research question.

There is evidence to suggest that the use of structured dysphagia assessment and management protocols may be effective in improving outcomes in patients with dysphagia. Historically, little emphasis has been placed on the identification and

management of silent aspiration in such protocols. The incorporation of CRT into a dysphagia protocol may guide clinicians towards optimum feeding management decisions as well as identify patients at risk of silent aspiration who may benefit from further instrumental assessment of swallowing.

7.2.2. Hypotheses.

1) Patients who are managed under the Dysphagia in Stroke Protocol (DiSP) will have a significantly reduced rate of pneumonia over three months, compared to an historical cohort.
2) Patients who are managed under the DiSP will have a significantly reduced length of hospital stay, compared to an historical cohort.
3) Route of nutrition/hydration intake at discharge will differentiate patients who are managed under the DiSP from an historical cohort, with significantly more patients in the latter group requiring an alternative route of intake.
4) Diet restriction at discharge (e.g. thin vs. thick fluids; modified diet texture) will differentiate patients who are managed under the DiSP from an historical cohort, with significantly more patients in the latter group requiring some form of diet restriction.
5) Patients who are managed under the DiSP will show a significantly reduced rate of re-admission for chest infection compared to an historical cohort within the first three months post-recruitment.

7.2.3. Rationale.

Post-stroke pneumonia represents a significant health issue and is strongly associated with poor patient outcomes including mortality. In addition, the costs associated with treatment increase dramatically when pneumonia accompanies stroke. Although

pneumonia is multi-factorial, silent aspiration (aspiration in the absence of an observable cough response) has been implicated in the pathogenesis of pneumonia. Cough reflex testing provides information about a patient's reflexive cough response and silent aspiration risk. However, in the absence of a defined management protocol, the addition of the CRT into clinical routine does not improve patient outcomes (Miles et al., 2013a) suggesting that adherence to a protocol may be as important as the CRT itself. Standardising patient management has been shown to improve overall patient outcomes in many fields of medicine. The use of CRT combined with a standardised assessment and management protocol may be more effective in improving outcomes in a dysphagic stroke population compared to the use of CRT alone.

7.2.4. Significance.

Incorporating cough reflex testing into a standardised patient management protocol will improve clinicians' abilities to make appropriate feeding management decisions. By identifying patients at high risk of silent aspiration early in the course of admission, completing instrumental swallowing assessments and following a protocol that bases feeding management on assessment findings, patients who are at risk of aspiration pneumonia can be managed appropriately, adverse outcomes can be avoided and hospitalisation costs can be lowered.

7.2.5. Proposed study.

The aim of this study is to evaluate the effect of a dysphagia assessment and management protocol that incorporated cough reflex testing in patients with acute stroke. The cough reflex test will be performed using the facemask method more suited to patients with neurological impairment and citric acid doses that are based on normative and validated data. Two hundred and eighty four patients will be recruited

to the study and will be managed according to the DiSP. Clinically-relevant outcomes will be measured at three months post-stroke and compared with 148 historical patients whose management included cough reflex testing but no protocol. The primary outcome of interest is aspiration pneumonia.

7.3. Study III: The Relationship between Oral Bacteria, Cough Reflex Sensitivity and Aspiration Pneumonia in Patients with Acute Stroke

7.3.1. Research question.

Patients with acute stroke and dysphagia are at increased risk for colonisation by potential respiratory pathogens such as *S. mitis, S. pnuemoniae, S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae.* This may be because of hemiplegia/hemiparesis and a sudden change to oral hygiene routines, dependence on others for oral hygiene, reduced consciousness, reduced salivary flow as well as other non-stroke-specific factors such as increased age, poor dental health and other co-morbidities. These patients are also at increased risk of pneumonia due to the aspiration of food/fluid/secretions that may be contaminated with pathogenic bacteria and reduced reflexive cough sensitivity. Despite this, no studies to date have documented changes in oral bacteria in the acute stages of stroke in patients with dysphagia. Do these changes exist and are they related to pneumonia in this population? Improving oral hygiene has been shown to improve reflexive cough sensitivity in elderly rest-home residents, but whether this is due to reduced oral bacteria or another mechanism is unclear. This can be clarified by answering the question: is there a relationship between oral bacteria and cough reflex sensitivity?

7.3.2. Hypotheses.

1) There will be a strong, positive correlation between prevalence of *S. mitis, S. pnuemoniae, S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae* isolated from patients' saliva samples and patients' cough reflex threshold levels.
2) Prevalence of *S. mitis, S. pnuemoniae, S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae* isolated from saliva samples and patients' cough reflex threshold levels will be independent predictors of aspiration pneumonia.
3) Numbers of *S. mitis, S. pnuemoniae, S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae* isolated from saliva samples will increase in proportion to the number of days a patient is hospitalised.
4) Numbers of *S. mitis, S. pnuemoniae, S. aureus, E. coli, P. aeruginosa* and *K. pneumoniae* isolated from saliva samples will be lowest at 30 days post-stroke.

7.3.3. Rationale.

To date, the relationship between oral bacteria, cough reflex sensitivity and aspiration pneumonia in patients with acute stroke and dysphagia is undocumented. Clarification of this relationship is needed before large-scale studies addressing oral care and recovery of cough reflex sensitivity in this population can be undertaken.

7.3.4. Significance.

Findings from this study will contribute towards a greater understanding of the role of oral bacteria in aspiration pneumonia. By focussing on a patient population who are at increased risk of silent aspiration and subsequent pneumonia, results of this study will represent the first step towards inform the optimal allocation of healthcare resources,

addressing oral care during the acute recovery phase of stroke and potentially decreasing the rates of aspiration pneumonia.

7.3.5. Proposed study.

The aim of this study is to describe the relationships between pathogenic oral bacteria, cough reflex sensitivity and aspiration pneumonia over three time points in patients with acute stroke and dysphagia. One hundred and two patients will be enrolled in the study. Each will provide saliva samples and undergo cough reflex threshold testing at three time points: on admission to hospital, at discharge from the acute stroke ward and at one month post-stroke. In addition, patients' medical notes will be reviewed for symptoms of aspiration pneumonia. Molecular testing will be used to identify the presence and prevalence of six target bacteria known to cause aspiration pneumonia in the saliva samples. The outcomes of interest are cough reflex sensitivity, presence/prevalence of target bacteria and diagnosed aspiration pneumonia.

Part B: Experimental Studies

Chapter 8. Diurnal Variation in Cough Reflex Testing

8.1. Research Aim

Diurnal variation in cough reflex testing is considered to be major confounding variable due to significant differences in cough reflex thresholds measured in the morning compared to the afternoon (Pounsford & Saunders, 1985). The evidence for this comes from a single historical study that used a vital-capacity/mouthpiece method and did not control for the possible confounding effect of increased oral bacteria throughout the day. The aim of this study was to investigate diurnal variation in cough reflex testing using CRT methods commonly used in clinical assessment and controlling for oral bacteria. Participants underwent repeated cough reflex sensitivity testing in the morning and the afternoon and results were compared. Oral bacteria were controlled for by participants brushing their teeth prior to each CRT.

8.2. Materials and Methods

8.2.1. Study design.

Local ethical approval was received for this prospective experimental study (University of Canterbury Human Ethics Committee reference HEC 2014/36). Informed written consent was given prior to commencement of data collection.

Participants provided data in two assessment sessions: one in the morning (between 9am – midday) and one in the afternoon (between 2 – 5pm). The order of testing was randomised and occurred on either the same day (i.e. morning, afternoon) or consecutive days (i.e. afternoon, morning). There was a minimum of three hours between sessions, with a mean of ten hours. In order to control for the potential confounding effect of oral bacteria on cough reflex thresholds (Watando et al., 2004), participants were instructed to brush their teeth using a toothbrush and water for two

minutes at the start of each session.

8.2.2. Participants.

Intially, 28 young, healthy participants were studied. An additional 25 participants were recruited in an attempt to increase statistical power so that the final dataset consisted of 53 participants (27 males, 26 females, age range = 19 – 37 years). Participants had no history of respiratory disease (e.g. severe asthma, chronic obstructive pulmonary disease), gastroesophageal reflux, neurological disorder (e.g. stroke, brain tumour, traumatic brain injury), chest infection within the past eight weeks or tobacco smoking in the past six months. Participants had not been previously exposed to CRT and none reported having a current dental infection or currently taking antibiotics, painkillers, cough syrup or ACE inhibitor drugs.

8.2.3. Cough reflex threshold testing.

Citric acid diluted in 0.9 % sodium chloride was prepared at twelve doses starting from 0.1 mol/L (1.92 %) and increasing in 0.1 mol/L increments up to 1.2 mol/L (23.06 %). This range was based on normative data (Monroe et al., 2014) which state that 96 % of people reach a suppressed cough threshold by 1.2 mol/L using a tidal breathing/face mask method. This range also includes the doses reported by Pounsford and Saunders (1985) as sensitive enough to detect diurnal variation in airway sensitivity and reflect current clinical methods (Kallesen, Psirides, & Huckabee, 2015; Miles, McLauchlan, & Huckabee, 2014)

CRT was performed using a tidal breathing/face mask method. Citric acid was delivered via a facemask (Hudson Micro Mist Nebuliser Model 41893, Standard Connector & Adult Mask, Hudson RCI, Durham, North Carolina, USA) placed over the nose and mouth. The facemask was connected to a Turboneb 2 Nebuliser (Clement Clarke International Limited, Harlow, UK) with an obstructed flow rate of

6.6 L/minute. As different aerosols were presented for up to fifteen seconds, participants were instructed to "breathe normally through your mouth. Try not to cough". The suppressed cough threshold (SCT) (as opposed to natural cough threshold) was chosen as the primary outcome measure, as this is considered to more closely approximate a true reflexive cough (Hegland et al., 2012; Monroe et al., 2014). Participants were blinded to the concentration of doses.

Initially, a placebo dose of 0.9 % sodium chloride was presented to accommodate the participants to the presentation of nebulised air. Up to twelve citric acid doses were presented in progressively higher concentrations, with placebo doses randomly interspersed throughout testing to increase challenge blindness and prevent tachyphylaxis (Morice, 1996). Each citric acid dose was presented up to three times, with at least 30 seconds between trials to prevent tachyphylaxsis (Morice et al., 2007). Cough response was considered positive if two or more consecutive coughs were triggered (C2 response threshold) on two out of three trials. The lowest concentration of citric acid that elicited a cough response was considered to be the SCT. All testing was video-recorded for reliability purposes.

8.2.4. Data analysis.

Statistical analyses were completed using SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Mac, Version 23.0. Armonk, New York, USA). An *a priori* sample size of 28 participants was calculated for an estimated effect size of 0.4 and 90 % statistical power ($p < .05$) based on the Wilcoxon signed-rank test. However, *post hoc* data analysis revealed low statistical power ($1 - \beta = 0.16$). Using the effect size calculated from the first analysis, a second Wilcoxon signed-rank test indicated that by increasing the sample size to $N = 53$, statistical power would increase and the chances of a Type II error would be reduced. Statistical significance was set at a level

of $p < .05$ (two-tailed). Related-samples Wilcoxon signed-rank tests were conducted to compare SCTs measured in the morning versus the afternoon and to compare participants' first SCT compared to their second SCT. Participants who did not cough at the highest citric acid dose were coded as having a SCT of 1.3 mol/L. A simple linear regression was used to predict SCT based on time of day (coded as hours since midnight). Intra-rater reliability was estimated by the primary researcher re-analysing 20 % ($n = 11$) of participants' recordings. The same 20 % samples were also used to estimate inter-rater reliability by two independent raters. Single measure intraclass correlation coefficients (ICC) were used for analysis.

8.3. Results

Morning SCTs (mean = 0.6 mol/L, $SD = 0.49$) were not different from afternoon SCTs [(mean = 0.6 mol/L, $SD = 0.46$), $p = .16$, $T = 101$, $r = -0.14$, $1 - \beta = 0.29$] (Figure 1). There were no gender differences, with male participants' morning SCTs (mean = 0.8 mol/L, $SD = 0.49$) not different from afternoon SCTs [(mean = 0.7 mol/L, $SD = 0.47$), $p = .19$, $T = 27$, $r = -0.18$]. Similarly, female participants' morning SCTs (mean = 0.5 mol/L, $SD = 0.47$) did not differ from afternoon SCTs [(mean = 0.4 mol/L, $SD = 0.44$), $p = .79$, $T = 30$, $r = -0.04$].

Regardless of time of day, SCTs from the first exposure (mean = 0.5 mol/L, $SD = 0.47$) were lower than the second exposure [(mean = 0.6 mol/L, $SD = 0.48$), $p = .01$, $T = 247$, $r = 0.27$, $1 - \beta = 0.77$] (Figure 2). This order effect was present for females [$p = .04$, $T = 56$, $r = 0.28$] but not males [$p = .06$, $T = 73$, $r = 0.26$].

Eleven participants (21 %) failed to trigger a SCT during either assessment. When these individuals were removed from the dataset, there continued to be no difference in morning SCTs (mean = 0.4 mol/L, $SD = 0.39$) compared to afternoon SCTs [(mean = 0.4 mol/L, $SD = 0.30$), $p = .16$, $T = 101$, $r = -0.14$]. Initial SCTs

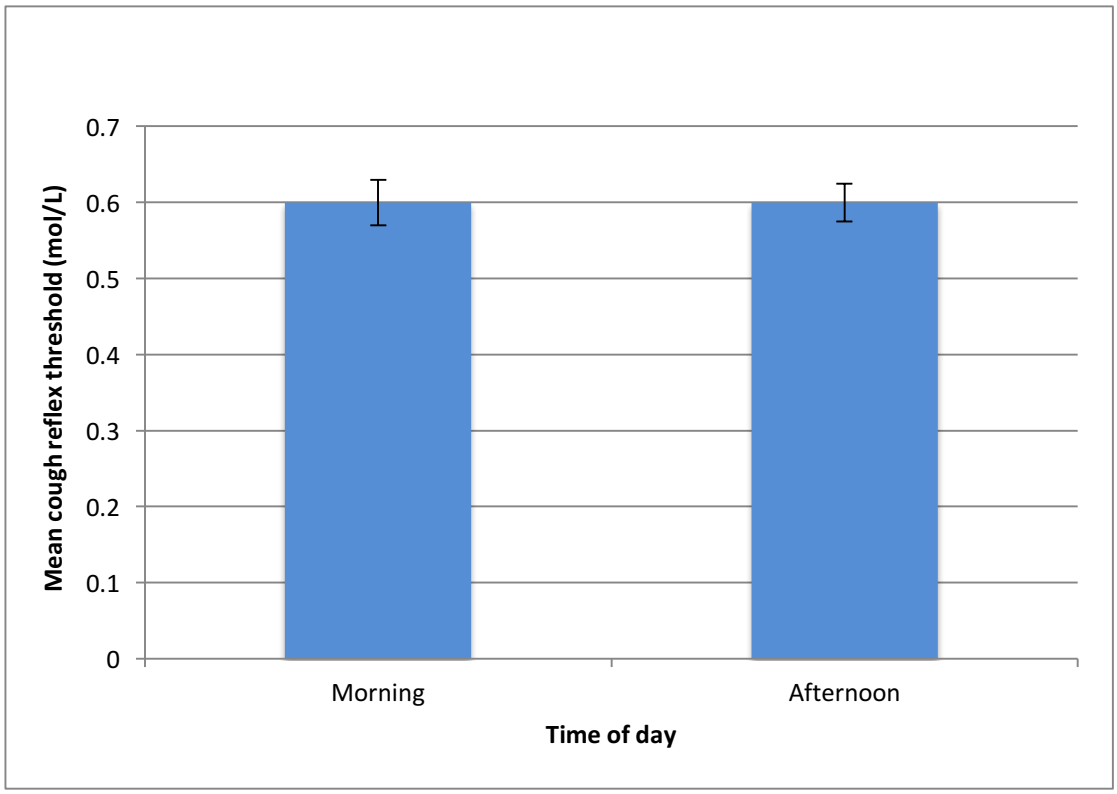


Figure 1. Cough reflex thresholds measured in the morning and the afternoon. Means and 95 % confidence intervals are depicted.

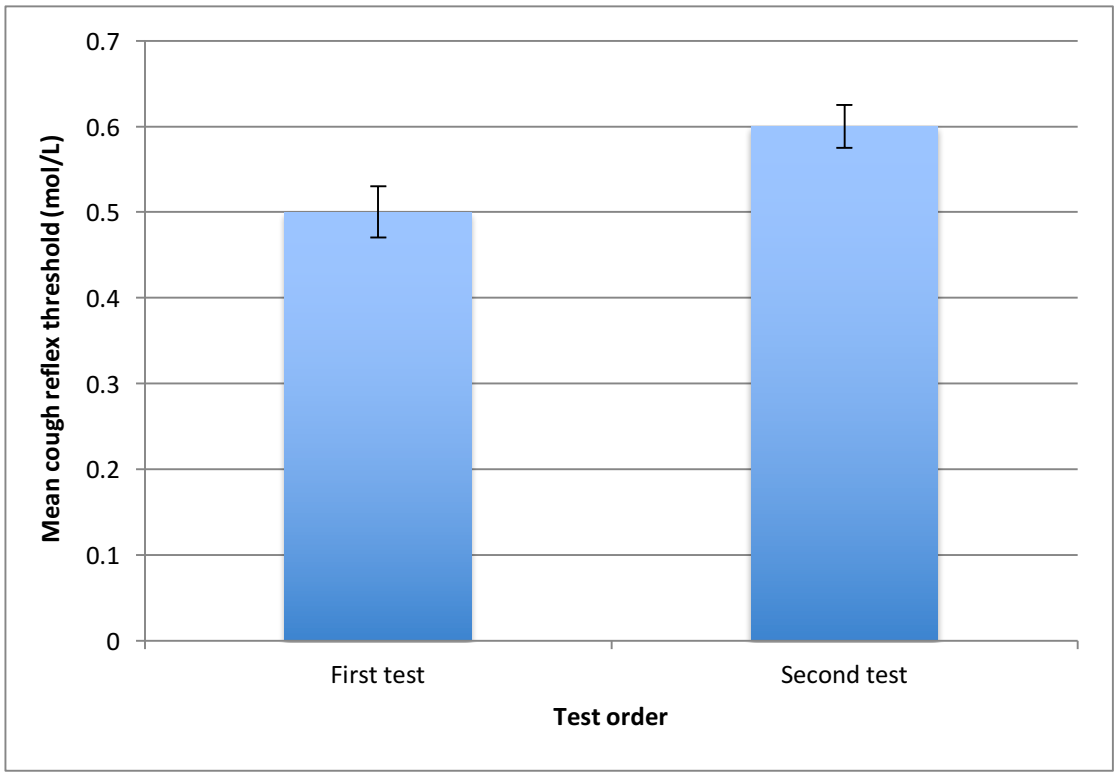


Figure 2. Cough reflex thresholds from the first and second tests. Means and 95 % confidence intervals are depicted.

(mean = 0.4 mol/L, *SD* = 0.31) continued to be lower than second SCTs [(mean = 0.4 mol/L, *SD* = 0.38), $p = .01$, $T = 247$, $r = 0.27$].

When time of day was analysed as a continuous variable (i.e. hours since midnight), no relationship to SCT was found [$F(1, 104) = .01$, $p = .93$]. The overall model fit was poor [$R^2 < .01$]. Time of day was not a significant predictor of SCT [$\beta = -0.01$, $p = .93$].

Intra-rater reliability of cough reflex sensitivity was excellent (Cicchetti, 1994), with a single measure ICC of 0.99. Inter-rater reliability was also excellent with an ICC of 0.99.

Chapter 9. Evaluation of the Dysphagia in Stroke Protocol in Patients with Acute Stroke

9.1. Research Aim

The use of standardised patient management protocols is strongly advocated in the literature. Despite this, there is a lack of high-quality evidence into the use of dysphagia management protocols in the acute stroke population. The aim of this study was to evaluate the effect of the DiSP: a protocol that incorporated cough reflex testing, instrumental assessment and prescriptive clinical guidelines around the resumption of oral intake in acute stroke patients. Clinically-relevant outcomes from patients managed according to the DiSP were measured and compared to patients who had received CRT in the absence of a prescribed management protocol.

9.2. Materials and Methods

9.2.3. Study design.

This study was registered with the Australian New Zealand Clinical Trials Registry (Trial Id: ACTRN12613000489796). Regional and locality ethical approval was received (Northern A Health and Disability Ethics Committee reference 13/NTA/111, University of Canterbury Human Ethics Committee reference HEC 2013/105) in conjunction with the Canterbury District Health Board Te Komiti Whakarite (Maori Ethics Committee).

The study was a prospective-retrospective cohort study. Miles et al. (2013a) compared pneumonia-related outcomes in 311 patients admitted to four metropolitan hospitals (Hospitals A, B, C, D) with acute stroke and suspected dysphagia. Patients were randomly assigned to either a control group who received a standard clinical

evaluation (n = 163) or an experimental group who received CRT in addition to standard clinical evaluation (n = 148). For the present study, 284 patients were prospectively recruited from a single metropolitan hospital (Hospital A). Patients had a diagnosis of stroke and suspected dysphagia and were managed using the DiSP: a standardised dysphagia assessment and management protocol. These patients were compared to the experimental group from Miles et al. (2013a) who had received CRT but no standardised management protocol.

9.2.4. Patient selection.

Patients who had been referred to SLT for swallowing evaluation were recruited for both studies. For details of recruitment see Figure 3. Patients who were referred for palliative swallowing advice were excluded, as these patients do not typically undergo complete swallowing assessments and prevention of pneumonia is not a priority in management. Twenty patients were excluded in the DiSP group; the reasons for exclusion are listed in Figure 3. For each patient who was excluded, a replacement patient was recruited.

9.2.5. Protocol.

The Dysphagia in Stroke Protocol (DiSP) was considered to be 'usual care' at Hospital A during the prospective arm of the study. The DiSP is conceptualised in Figure 4. Briefly, all patients referred for swallowing evaluation underwent a clinical swallowing assessment, including informal communication screening, cranial nerve examination and functional assessment of oral, pharyngeal and laryngeal substrates. CRT was completed prior to the assessment of oral intake. If the patient failed the CRT, they were recommended NBM and referred for a VFSS. If the patient passed the CRT, they proceeded to an evaluation of oral intake. The DiSP also guided clinicians in their interpretation of VFSS findings. If no aspiration was observed, the

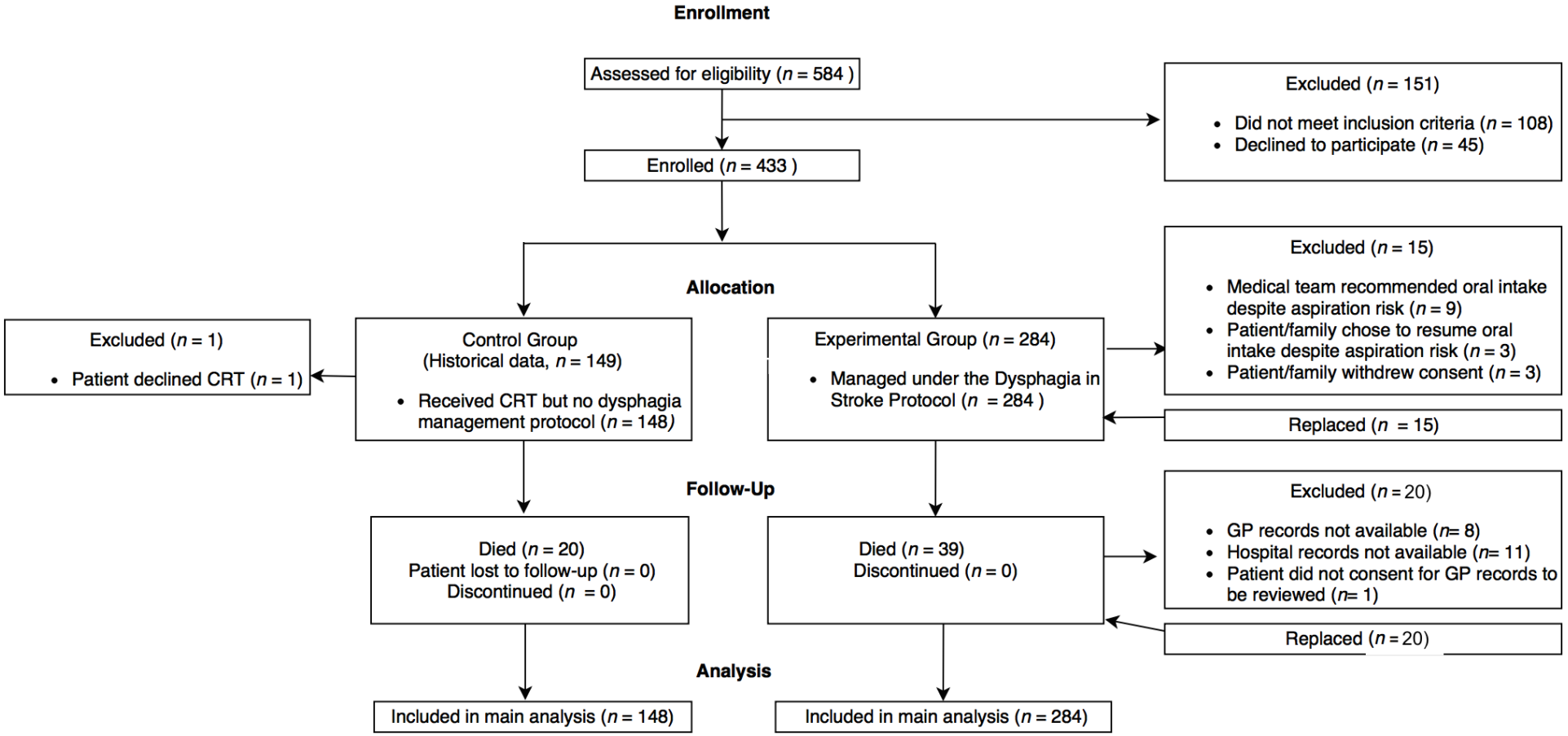


Figure 3. Details of Study Recruitment

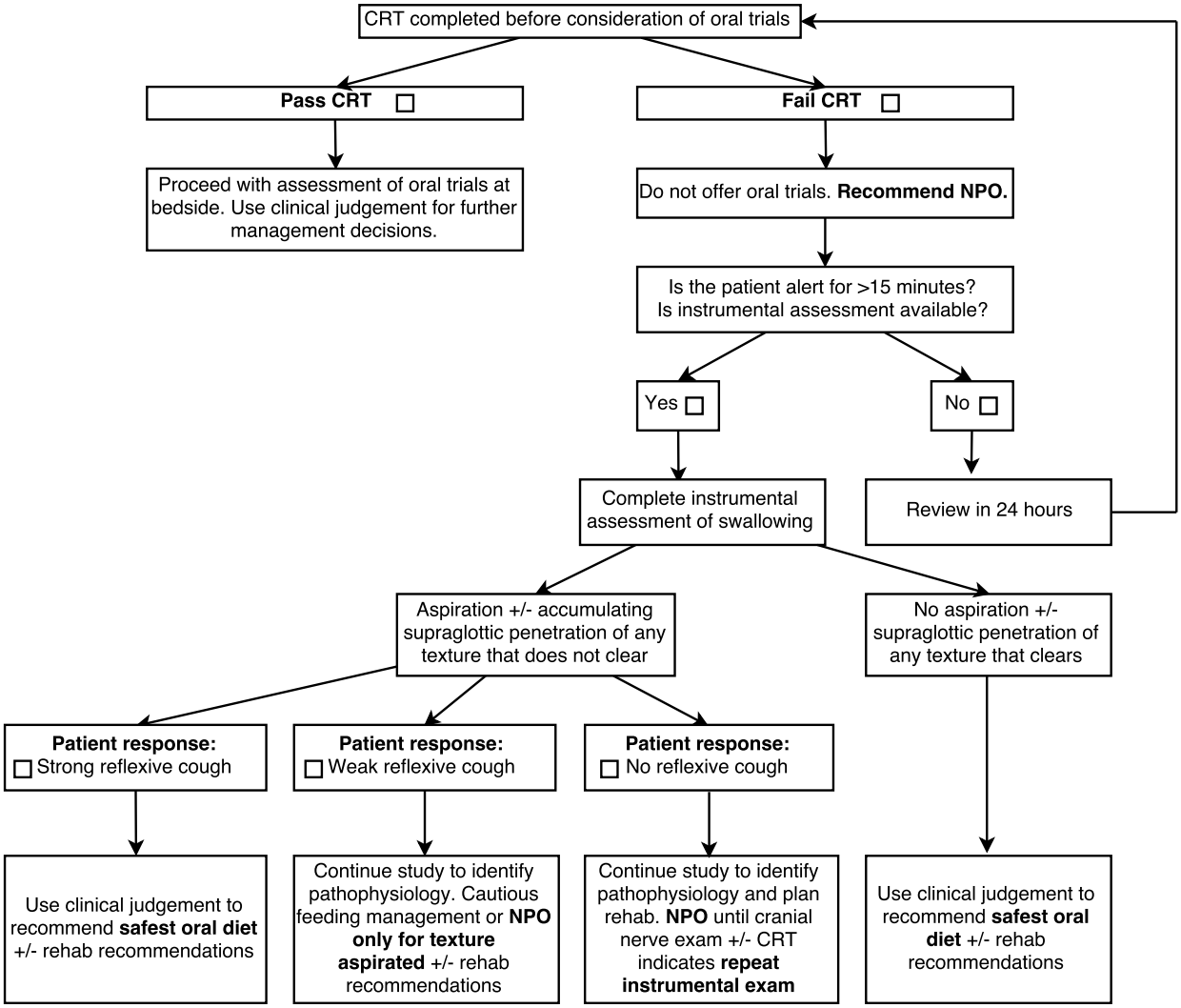


Figure 4. The Dysphagia in Stroke Protocol

patient was recommended an appropriate oral diet. If aspiration was observed, recommendations regarding oral intake were made according to the patient's ability to clear the aspirate.

9.2.5.1. Cough reflex testing.

CRT was performed based on the method described by Miles et al. (Miles et al., 2013a). Citric acid diluted in 0.9 % sodium chloride was prepared at two doses: 0.8 mol/L at which 90 % of healthy people produce evoked coughing (Monroe et al., 2014) and a higher dose of 1.2 mol/L at which 96 % of people can no longer suppress coughing (Monroe et al., 2014), considered to more closely represent a true reflex cough (Hegland et al., 2012). CRT was performed using a tidal breathing/face mask method. Citric acid was delivered via a facemask (Hudson Micro Mist Nebuliser Model 41893, Standard Connector & Adult Mask) placed over the nose and mouth. The facemask was connected to a Turboneb 2 Nebuliser (Clement Clarke International Limited) with an obstructed flow rate of 6.6 L/minute. Initially, a placebo dose of 0.9 % sodium chloride was presented to acclimate the patient to the presentation of nebulised air. Citric acid at a dose of 0.8 mol/L was then presented with the instruction to "breathe normally through your mouth. Cough if you need to". Citric acid was presented for up to 15 seconds, during which time coughing presence/absence and a subjective judgement of coughing strength (strong/weak) was noted. The test was repeated up to three times at the lower dose, with at least 30 seconds between presentations to prevent tachyphylaxis (Morice et al., 2007). Coughing response was considered positive if two or more consecutive "strong" coughs were triggered (C2 response threshold) on two out of three trials (Morice et al., 2007). The clinician then instructed the patient to "try not to cough" while administering the same low dose. If the patient was able to suppress coughing at 0.8

mol/L on two out of three trials, the test was repeated again using the higher dose at 1.2 mol/L.A patient was considered to pass the CRT if they produced strong coughing to 0.8 mol/L citric acid and could not suppress coughing at either 0.8 mol/L or 1.2 mol/L. A patient was considered to fail the CRT if they produced weak coughing to 0.8 mol/L or could suppress coughing at 1.2 mol/L.

Patients who passed the CRT proceeded to bedside evaluation of oral intake, with decisions regarding diet modification, supplemental nutrition/hydration and referral for instrumental assessment determined by the SLT based on clinical presentation on further examination and in conjunction with the multi-disciplinary team. Patients who failed the CRT remained non-oral and were immediately referred for a VFSS.

9.2.5.2. Diet management.

The DiSP also dictated diet management following VFSS. Diet recommendations were made on the basis of aspiration and the patient's response. Patients who presented with silent aspiration remained non-oral with alternative nutrition/hydration until recovery of the cough reflex (as determined by repeat CRT) or aspiration resolved (as determined by repeat VFSS). Patients who responded to aspiration with a weak cough response remained non-oral for the texture aspirated, with other textures recommended at the clinician's discretion. Patients who presented a strong cough response to aspirate were recommended the safest oral diet per the clinician's judgement. Similarly, patients with no aspiration were recommended the safest oral diet per the clinician's judgement.

9.2.6. Control group.

Patients in the historical control group underwent a bedside swallowing assessment, including informal communication screening, cranial nerve examination and

functional assessment of oral, pharyngeal and laryngeal substrates. CRT was completed prior to the assessment of oral intake, using the method described by Miles et al. (2013a). Subsequent management decisions were not prescribed and were left to the discretion of the clinician.

9.2.7. Outcome measurement.

The primary outcome of interest was the proportion of patients with AP within 3 months of recruitment. A diagnosis of AP was made on the basis of previously-described criteria, namely, the presence of three or more of the following: fever (>38 °C), abnormal chest examination (tachypnea [>22 breaths/minute], tachycardia, inspiratory crackles, bronchial breathing), productive coughing with purulent sputum, abnormal chest x-ray, arterial hypoxemia (PO_2 <70 mm Hg) and detection of a relevant pathogen (positive Gram stain and culture) (Mann et al., 1999). Secondary outcome measures included: mortality, length of hospital stay, proportion of patients readmitted to hospital with pneumonia within three months and place of residence at three months. Demographic information and medical histories were collected from medical records. Clinical decision parameters were also examined, including use of instrumental swallowing assessment, recommended route of intake at three months (oral versus non-oral) and recommended diet restriction at three months (altered food/fluid texture). The primary outcome measure and several of the secondary outcome measures were determined by a physician and documented in the medical records. Outcome measurement was conducted by the primary researcher (S.D.) via telephone or facsimile to participants, family members, residential care staff and/or general practitioners as well as a retrospective chart review at three months.

9.2.8. Data analysis.

Statistical analyses were completed using SPSS (IBM Corp.). After an evaluation by the clinical advisory board for this study, a relative risk reduction for pneumonia of 40 % was considered clinically significant and achievable. Using a binomial proportion test, an *a priori* sample size of 284 participants in the experimental group was calculated for an estimated effect size of 0.4 and 90 % statistical power ($p < .05$).

Binomial logistic regressions were performed to ascertain the effects of the DiSP protocol on the likelihood of 1) patients developing AP, 2) mortality, 3) pneumonia-related mortality, adjusted for confounding variables (age, gender, ethnicity, study site, cardiac and respiratory co-morbidities, previous stroke, lesion site and laterality, initial CRT result). Negative binomial regressions were completed to compare the mean length of stay (LOS) between groups. Patients who died during hospitalisation were excluded from LOS analysis. Multinomial logistic regressions were conducted to determine the effects of the DiSP on patients' place of residence at three months post-stroke, adjusted for confounding variables (gender, age, cardiac and respiratory co-morbidities, previous stroke and pre-stroke residence). Patients' pre-stroke place of residence was categorised as either 'independent' (i.e. patient lived in their own home) or 'dependent' (i.e. patient lived in a rest home or public hospital at the time of their stroke). Chi-square tests were conduced to compare the proportion of patients in each group who were living at home vs. residential care facility vs. hospital at three months. For comparisons involving the patient returning home, only patients who were living at home prior to the stroke were considered. Chi-square tests were also conducted to assess the associations between diet tolerance at three months and group. Diet tolerance was categorised as tube-fed with no/minimal oral intake, modified oral diet +/- supplemental tube feeding or normal diet. Patients who had

died (n = 38) or who were lost to follow-up (n = 33) were excluded from this analysis. An independent samples t-test was used in a subsample of patients who underwent a VFSS to measure differences in the number of days between the initial clinical assessment of swallowing and receiving the VFSS between the two groups.

9.3. Results

Four hundred and thirty-two patients were included in the analysis: 284 patients managed per the DiSP and 148 historical control patients. Control patients were recruited from four different hospitals; however, patients did not significantly differ in terms of age, gender, lesion site or laterality, history of stroke or respiratory comorbidities, pre-stroke independence, response to CRT or initial diet recommendations [$p > .05$].

The DiSP group and control group did not differ significantly on major demographic varables (Table 6). There were significant differences in ethnicity [$p < .001$], respiratory comorbidities [$p = .02$] and cardiac comorbidities [$p = .004$]. The groups also differed in terms of patients' initial responses to CRT: more patients passed their initial CRT in the DiSP group compared to the control group and more patients in the control group had a weak or absent cough response compared to the DiSP group [$p = .001$]. See Table 7 for a summary of outcomes between the DiSP and control groups.

Within the DiSP group, clinician compliance with the protocol was 90 %. Noncompliance with the DiSP was mainly due to the incorrect administration of the CRT (5 %), lack of referral for VFSS despite noting overt signs of aspiration on clinical examination (4 %) and inappropriate diet recommendations following VFSS (2 %). The decision was made to retain data from these participants in the analyses to

Table 6

Demographic Comparisons Between Experimental Group and Control Group

Demographics	DiSP (n = 284)	Control (n = 148)	p value
Age	Mean 76 years (SD = 12)	Mean 76 years (SD = 15)	.81
Male	143 (50 %)	78 (53 %)	.72
Ethnicity:			**< .001**
Caucasian	260 (92 %)	111 (75 %)	
New Zealand Maori	8 (3 %)	16 (11 %)	
Pacific Islander	7 (2 %)	13 (9 %)	
Other	11 (4 %)	8 (5 %)	
Hospital Site:			**< .001**
Hospital A	284 (100 %)	43 (29 %)	
Hospital B	0 (0 %)	37 (25 %)	
Hospital C	0 (0 %)	52 (35 %)	
Hospital D	0 (0 %)	16 (11 %)	
Comorbidities:			
Previous stroke	82 (29 %)	44 (30 %)	.89
Respiratory comorbidities	52 (18 %)	15 (10 %)	**.02**
Cardiac comorbidities	155 (55 %)	103 (70 %)	**.004**
Site of lesion:			.10
Supratentorial	241 (85 %)	127 (86 %)	
Infratentorial	30 (11 %)	11 (7 %)	

Mixed	4 (1 %)	1 (1 %)	
Not detected	9 (3 %)	8 (5 %)	
Laterality of lesion:			.41
Left	116 (41 %)	69 (47 %)	
Right	138 (49 %)	64 (43 %)	
Bilateral	16 (6 %)	15 (10 %)	
Not reported	14 (5 %)	0 (0 %)	
Independence upon admission:			.39
Residential care facility or public hospital	31 (11 %)	20 (14 %)	
Home	253 (89 %)	128 (86 %)	
Response to initial cough reflex test:			**.001**
Strong coughing (pass)	206 (73 %)	91 (61 %)	
Weak coughing (fail)	40 (14 %)	33 (22 %)	
Absent coughing (fail)	38 (13 %)	26 (18 %)	
Diet following initial assessment:			.55
Non-oral	103 (36 %)	43 (29 %)	
Modified oral	90 (32 %)	40 (27 %)	
Normal oral	91 (32 %)	22 (17 %)	

SD = standard deviation

Table 7

Comparison of Outcomes Between DiSP Group and Control Group

	DiSP	Control	*p* value
Pneumonia	29 (10 %)	41 (28 %)	**.03**
Mortality	39 (16 %)	20 (14 %)	.37
Pneumonia-related mortality	17 (6 %)	7 (5 %)	.50
Number of days in acute ward†	$\overline{x}$ = 6 days	$\overline{x}$ = 9 days	**<.001**
Number of days in hospital (acute + rehabilitation)†	$\overline{x}$ = 24 days	$\overline{x}$ = 32 days	**<.001**
Received an instrumental swallowing assessment	88 (31 %)	27 (18 %)	**.005**
Readmission to hospital for pneumonia	0 (0 %)	7 (5 %)	.99
Independence at 3 months post-assessment†			
Public hospital	9 (4 %)	41 (32 %)	**<.001**
Residential care facility	63 (29 %)	16 (13 %)	**<.001**
Home*	142 (71 %)	71 (63 %)	.17
Diet at 3 months post-assessment†			
Tube-fed	1 (0.5 %)	3 (2 %)	.15
Modified oral diet	54 (43 %)	40, 19 %)	**<.001**
Normal diet	172 (81 %)	70 (55 %)	**<.001**

†Excluding participants who had died or were lost to follow-up. *Excluding participants who were not living in their own home pre-stroke.

accurately represent the real-world clinical application of the DiSP.

9.3.1. Reducing complications of acute stroke.

9.3.1.1. Aspiration pneumonia.

There was a significant difference in the rate of AP between the DiSP group and the control group [adjusted odds ratio: 2.72, 95 % *CI* 1.08 – 6.85, *b* = 1.00, *p* = .03] (Figure 5). This result was not associated with whether a patient passed or failed their initial CRT [*p* = .19]. The logistic regression model significantly predicted the development of AP [$\chi^2(19) = 44.42$, $p = .001$]. Nagelkerke R^2 for the model with group as the only predictor was .08, indicating a small effect size (Cohen, 1988). However, the model did correctly classify pneumonia development in 84 % of cases. Nagelkerke R^2 increased to .17 with the addition of age, gender, ethnicity, study site, CRT result, lesion site and laterality and patient comorbidities to the model, suggesting that these variables accounted for an additional 9 % of the variance in the development of AP. Regression coefficients and standard errors can be found in Table 8.

9.3.1.2. Mortality.

There was no significant difference in overall mortality between the two groups [*p* = .37] (Figure 6). Within the control group, the presence of cardiac comorbidities was a significant predictor of mortality [adjusted odds ratio: 6.72, 95 % *CI* 1.16 – 38.98, *b* = 1.91, *p* = .03]. Within the DiSP group, there was a significant main effect of age, with increased age associated with an increased risk of mortality [odds ratio: 1.07, 95 % *CI* 1.02 – 1.12, *b* = .06, *p* = .005]. There was no significant effect of group on mortality due to pneumonia [odds ratio: 1.37, 95 % *CI* 0.45 – 4.17, *b* = 0.32, *p* = .50] (Figure 7). Nagelkerke R^2 for the model with group as the only predictor was .007. This increased

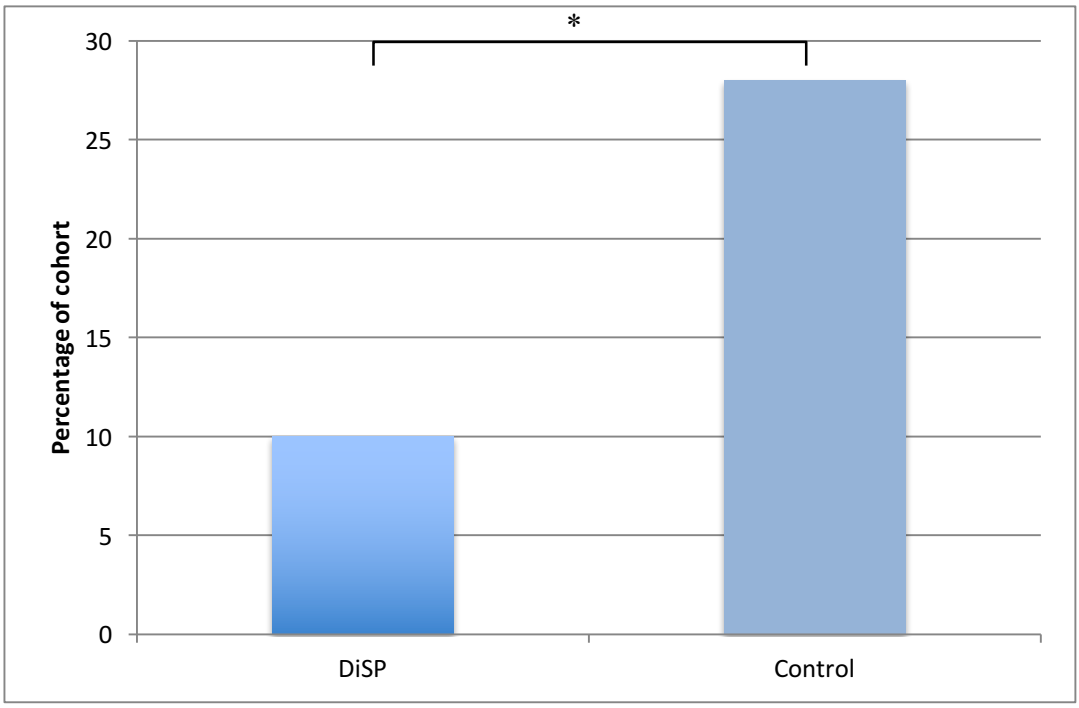


Figure 5. Percentage of patients in the DiSP and control groups who developed aspiration pneumonia. * $p = .03$.

Table 8

Results from Logistic Regression Model Predicting Aspiration Pneumonia

		95 % *CI* for Odds Ratio		
	B (SE)	Lower	Odds Ratio	Upper
Constant	-3.43 (1.37)		0.03	
Control group vs. DiSP	1.00* (0.47)	1.08	2.72	6.85
Age	0.02 (0.01)	0.99	1.02	1.04
Male vs. Female	0.10 (0.30)	0.61	1.10	1.99
Ethnicity				
Maori vs. Other	0.63 (0.69)	0.48	1.88	7.28
Pacific Island vs. Other	-0.21 (0.82)	0.16	0.81	4.02
NZE vs. Other	0.56 (0.47)	0.69	1.74	4.40
Lesion site:				
Supratentorial vs. Mixed	-0.19 (1.19)	0.08	0.83	8.57
Infratentorial vs. Mixed	-1.28 (1.30)	0.02	0.28	3.57
Not reported vs. Mixed	0.21 (1.48)	0.07	1.23	22.45
Lesion laterality:				
Right vs. Not reported	0.54 (0.90)	0.30	1.71	9.93
Left vs. Not reported	0.47 (0.90)	0.28	1.61	9.29
Bilateral vs. Not reported	1.81 (1.07)	0.75	6.10	49.65
Previous stroke	5.26 (0.30)	0.67	1.21	2.18
Respiratory comorbidities	1.85 (.38)	0.81	1.72	3.62

Cardiac comorbidities	2.76 (0.33)	0.76	1.44	2.72

Note. DiSP = Dysphagia in Stroke Protocol, NZE = New Zealand European, * $p < .05$

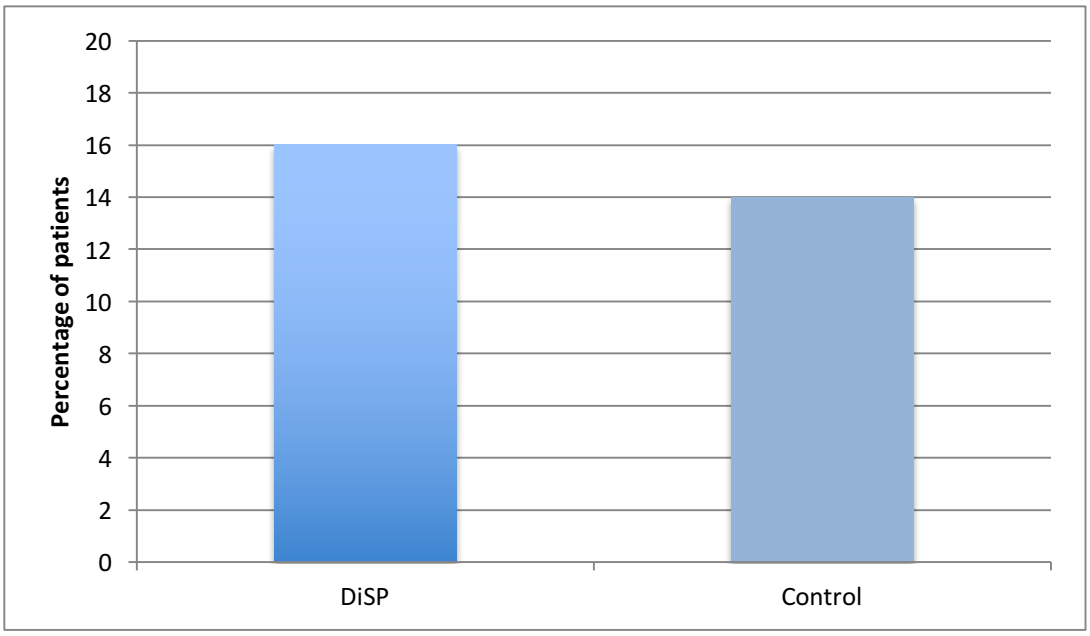


Figure 6. Percentage of patients in the DiSP and control groups who died within three months of stroke. The difference between the two groups is not statistically significant [$p > .05$].

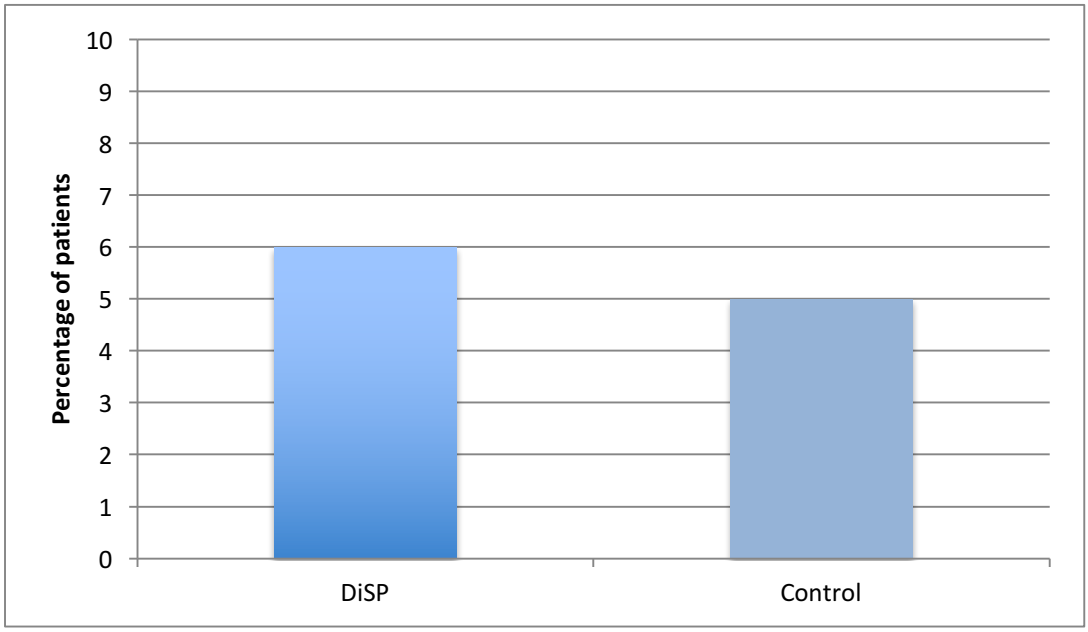


Figure 7. Percentage of patients in the DiSP and control groups who died as a result of pneumonia within three months of stroke. The difference between the two groups is not statistically significant [$p > .05$].

to .32 with the addition of age, gender, ethnicity, study site, lesion site and laterality and patient comorbidities to the model, suggesting that these variables accounted for an additional 31 % of the variance in pneumonia-related mortality. Other causes of death are listed in Table 9.

Within the DiSP group, further analysis was undertaken of patients who died as a result of AP (n = 16). Ten patients (63 %) in this group passed their initial CRT and six patients (38 %) failed. Three of sixteen patients (19 %) were not immediately diagnosed with stroke and were provided oral nutrition and hydration prior to an assessment of swallowing. Two of these patients passed their CRT and one patient failed. Two patients (13 %) were not managed strictly per the DiSP. One of these patients could not complete the full CRT due to difficulties following instructions but was considered to have passed the CRT. One patient was not referred for VFSS until the sixth SLT review, despite failing the CRT and presenting overt signs of aspiration as documented in five prior SLT reviews. Four patients (25 %) were identified as potential silent aspirators yet, despite clinical efforts, developed AP. The remaining seven patients (44 %) were not identified as at risk of silent aspiration.

9.2.1.3. Length of hospitalisation.

Patients in the control group spent significantly more days on an acute stroke ward compared to patients in the DiSP group [adjusted odds ratio 1.49, 95 % CI 1.31 – 1.71, b = .40, p < .001] (Figure 8). Across both groups, male patients also spent significantly longer on the acute ward [$\overline{x}$ = 7 days] compared to female patients [$\overline{x}$ = 6 days, adjusted odds ratio 1.15, 95 % CI 1.32 – 1.01, b = .14, p = .04]. Control patients spent significantly more days in hospital (acute + rehabilitation [$\overline{x}$ = 32 days]) compared to DiSP patients [$\overline{x}$ = 24 days, adjusted odds ratio 1.42, 95 % CI 1.17 – 1.71, b = .35, p < .001]. Lesion laterality also predicted LOS [p < .001]. Specifically,

Table 9

Reported Causes of Death other than Pneumonia

Reported Cause of Death	*n*
Stroke	15
Cardiac arrest/heart attack	5
Unknown/not reported	4
Septicaemia	2
Stroke, exhaustion coma, cerebral hypoxia	1
Stroke, primary lung neoplasm	1
Stroke, atrial fibrillation	1
Intracerebral haematoma	1
Pulmonary oedema, post-stroke seizures	1
End stage congestive heart failure, septicaemia, limb ischaemia	1
Urosepsis	1
Cancer	1
Renal failure	1
Ulcers	1

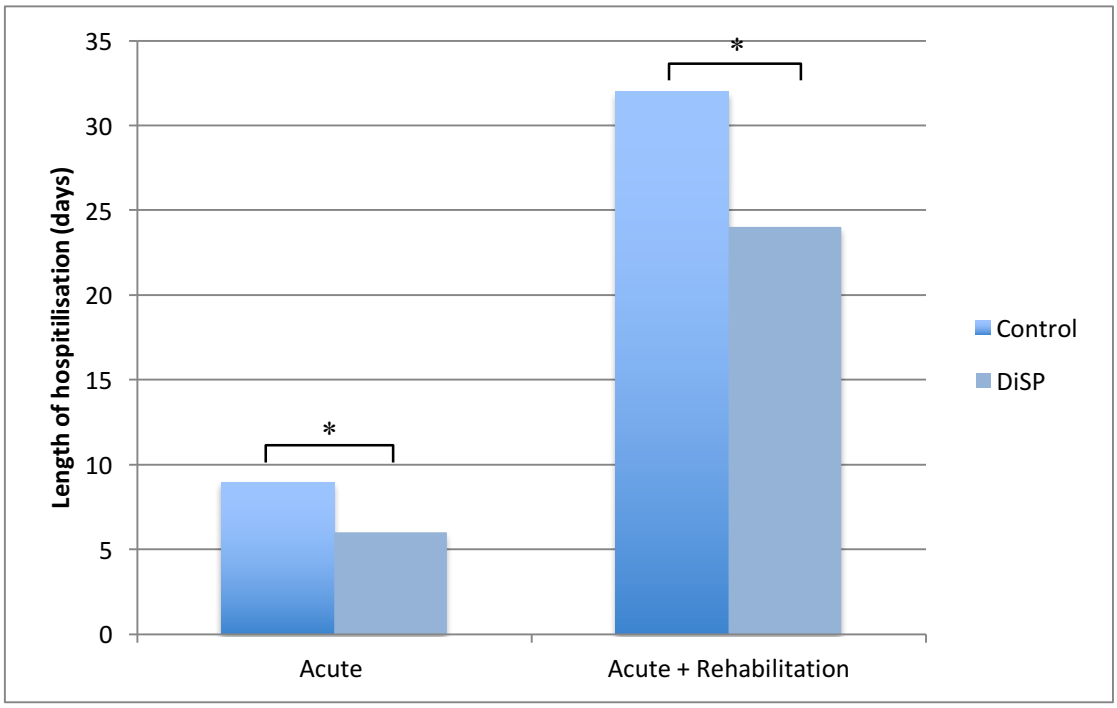


Figure 8. Length of acute hospitalisation and total hospitalisation for patients in the DiSP and control groups. * $p < .001$.

patients for whom lesion laterality was not reported spent the least amount of days in hospital [$\overline{x}$ = 9 days], followed by patients with bilateral lesions [$\overline{x}$ = 19 days] and patients with left- or right-sided lesions [$\overline{x}$ = 27 days, $\overline{x}$ = 28 days].

9.2.1.4. Post-stroke independence.

By three months, the majority of patients in both the DiSP and control groups had returned to their own home (Figure 9). Although greater numbers of patients in the DiSP group had returned home compared to the control group, this difference was not statistically significant [$\chi^2(1) = 2.22$, $p = .17$]. In the DiSP group, significantly more patients were in a residential care facility compared to patients in the control group [$\chi^2(1) = 12.46$, $p < .001$] (Figure 9). Of patients who were still in hospital, or had returned to hospital, at three months, significantly more were from the control group compared to the DiSP group [$\chi^2(1) = 50.51$, $p < .001$] (Figure 9). The best predictors of post-stroke place of residence were group, age and pre-stroke place of residence.

Compared to patients in the DiSP group, patients in the control group were 11.66 times more likely to be in hospital rather than home [adjusted odds ratio, 95 % CI 5.38 – 25.00, $p < .001$] and 23.81 times more likely to be in hospital rather than a rest home at three months [adjusted odds ratio, 95 % CI 9.62 - 58.82, $p < .001$]. Older patients were more likely to be in hospital or in a rest home compared to younger people [$p < .01$]. Patients who were independent (i.e. in their own home) pre-stroke were 12.35 – 26.32 times more likely to return home rather than reside in hospital or rest home compared to patients who were dependent (i.e. resided in a rest home or hospital) pre-stroke [$p < .001$].

9.2.1.5. Pneumonia-related hospital readmissions.

No patients in the DiSP group were readmitted to hospital with pneumonia during the

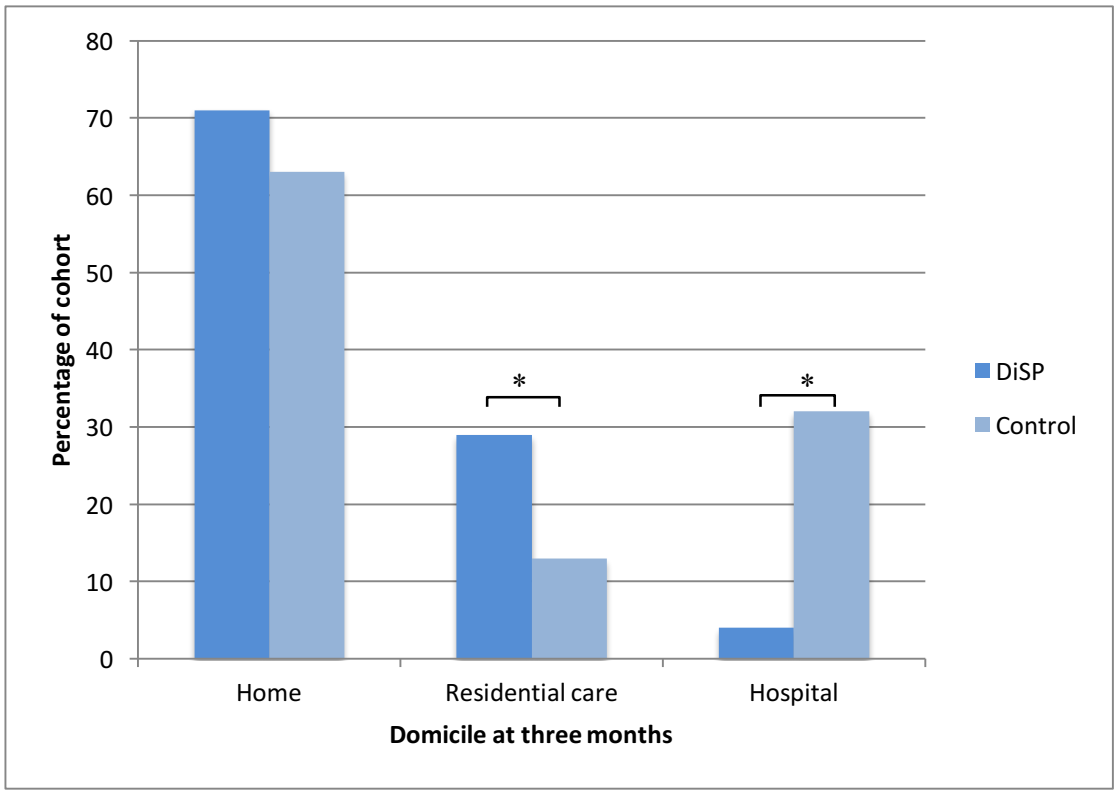


Figure 9. Percentage of patients in the DiSP and control groups who were living at home, in residential care or hospital at three months post-stroke. * $p < .001$.

study period, compared to seven patients (5 %) in the control group. The regression model significantly predicted readmission to hospital with pneumonia [$\chi^2(18)$ =36.93, p =.005]. Nagelkerke R^2 for the model with group as the only predictor was .23, indicating a small effect (Cohen, 1988). This increased to .49 with the addition of age, gender, ethnicity, study site, lesion site and laterality and patient comorbidities to the model, suggesting that these variables accounted for an additional 26 % of the variance in readmission to hospital with AP. However, no significant main effects emerged for any of the predictor variables.

9.2.1.6. Post-stroke oral feeding status.

At three months post-stroke, there was no association between group and tube-feeding [p = .15]. One patient in the DiSP group (3 %) was tube-fed at three months compared to three patients (2 %) in the control group. There were significantly more patients on a modified oral diet in the control group (43 %) compared to the DiSP group (19 %) [$\chi^2(1) = 22.42$, p <.001]. Significantly more patients were on a normal diet (81 %) in the DiSP group compared to the control group (55 %) [$\chi^2(1) = 25.48$, p <.001] (Figure 10).

9.2.1.7. Changes in clinical practice.

There was a significant increase in the rate of referral for instrumental swallowing assessment, with DiSP patients 2.02 times more likely to be referred for instrumental assessment compared to control patients [adjusted odds ratio, 95 % *CI* 1.24 – 3.30, b = .70, p = .005]. Among those patients who underwent a VFSS, the average time between undergoing an initial clinical swallowing evaluation and receiving a VFSS was 2.3 days (*SE* = 0.46) for patients in the DiSP group, compared to 8.5 days (*SE* =1.67) for patients in the control group. This difference was statistically significant

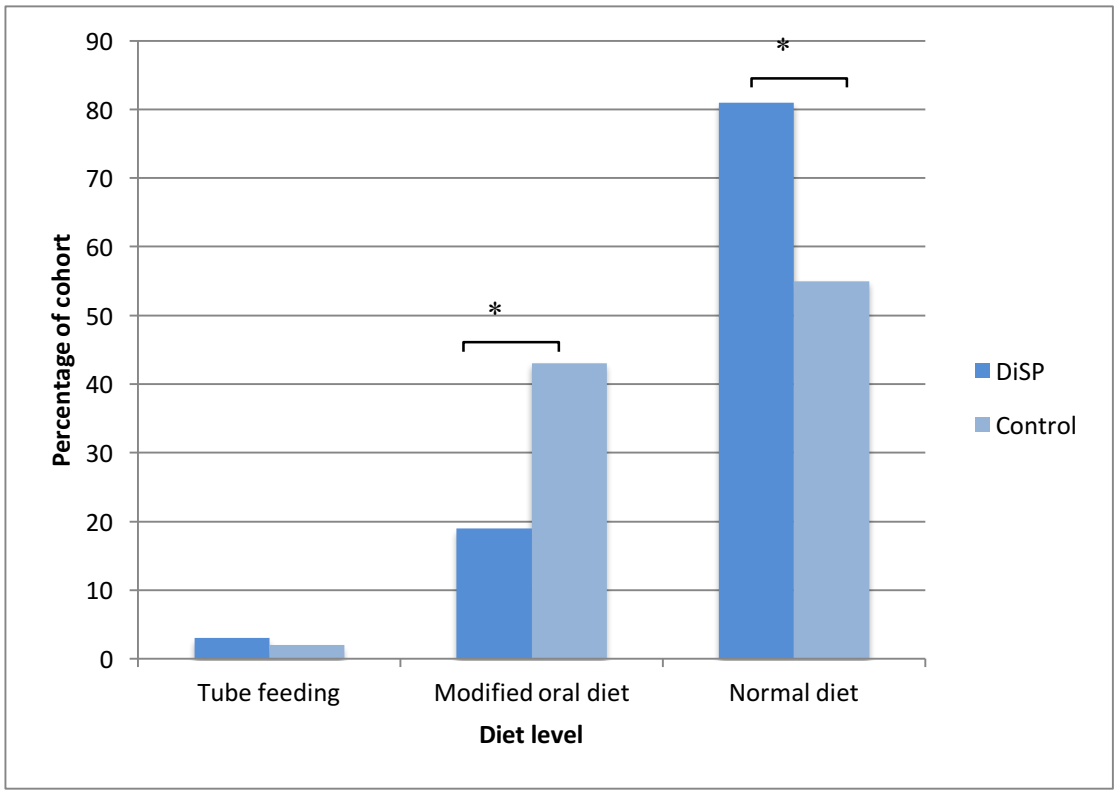


Figure 10. Percentage of patients in the DiSP and control groups who were tube-fed, eating a modified oral diet or a normal diet at three months post-stroke. * $p < .001$

and represented a medium-sized effect (Cohen, 1988) [$t(30) = 3.57$, $p = .001$, $r = .55$]. In the control group, three out of eight patients (38 %) who developed pneumonia were referred for instrumental assessment after the diagnosis of pneumonia was made. By comparison, five out of 15 patients (33 %) in the DiSP group who developed pneumonia received an instrumental swallowing assessment after the development of pneumonia. However, it should be noted that in four out of five patients pneumonia was suspected/confirmed at the time of admission to hospital. In the control group, the referral rate for VFSS following a failed CRT was 46 % compared to 95 % in the DiSP group.

Chapter 10. The Relationship Between Oral Bacteria, Cough Reflex Sensitivity and Aspiration Pneumonia in Patients with Acute Stroke

10.1. Research Aim

Despite known relationships between oral bacteria and pneumonia, there are few documented reports of oral bacteria in patients with acute dysphagia. This population are particularly at risk for developing pneumonia in the early stages of stroke. A link between oral bacteria levels and cough reflex sensitivity has been suggested, but to date it remains unknown whether this relationship exists. The aim of this research was to document the relationships between oral bacteria, cough reflex sensitivity and aspiration pneumonia in patients with acute stroke and dysphagia. Oral bacteria samples were measured at three points in time and compared to measures of patients' cough reflex sensitivity and the occurrence of aspiration pneumonia.

10.2. Materials and Methods

10.2.1. Study design.

Regional and locality ethical approval for this study was received (Southern Health and Disability Ethics Committee reference 13/STH/121, University of Canterbury Human Ethics Committee reference HEC 2013/132) in conjunction with the Canterbury District Health Board Te Komiti Whakarite (Maori Ethics Committee). Consecutive patients ($N = 102$) were recruited from a metropolitan hospital between February 2014 and July 2015. Repeated measures were taken at baseline (within two days of stroke), at discharge from the acute stroke ward and at 30 days post-stroke.

10.2.2. Participant selection.

Patients admitted to an acute hospital unit with a diagnosis of stroke and referred to SLT for an assessment of swallowing were approached for participation in this study. Patients who were referred for palliative swallowing advice – defined as advice that does not actively prevent pneumonia – were excluded, as these patients do not typically undergo complete swallowing assessments. Patients who had undergone CRT within the past twelve hours were also excluded due to sterilising effects of citric acid on bacteria (Falconer et al., 2014). Participants all provided written consent to take part in this study (Appendix I). For patients with aphasia, an "aphasia-friendly" consent form and supportive language techniques were used (Appendix I). If patients were unable to provide written consent, consent by proxy was sought. For details of recruitment see Figure 11. Where there was attrition, participants were replaced with patients who met the inclusion criteria for the study (i.e. diagnosis of acute stroke, referred for SLT assessment of swallowing).

10.2.3. Data acquisition.

10.2.3.1. Measurement of specific oral bacteria.

Two saliva samples were collected at each measurement point and were used to measure oral bacteria. Samples were obtained using tubed, sterile rayon swabs (DrySwab™, Innovatek Medical Incorporated, Delta, British Columbia, Canada) inserted into the mouth and held in the sublingual cavity for 30 seconds. Samples were immediately placed in a sterile tube, sealed and placed inside a cooler bag until they could be transferred to a freezer for storage at -70 °C. Samples were identified by a code consisting of 1 – 3 digits and one letter.

To avoid any potential sterilising effects of citric acid on oral bacteria,

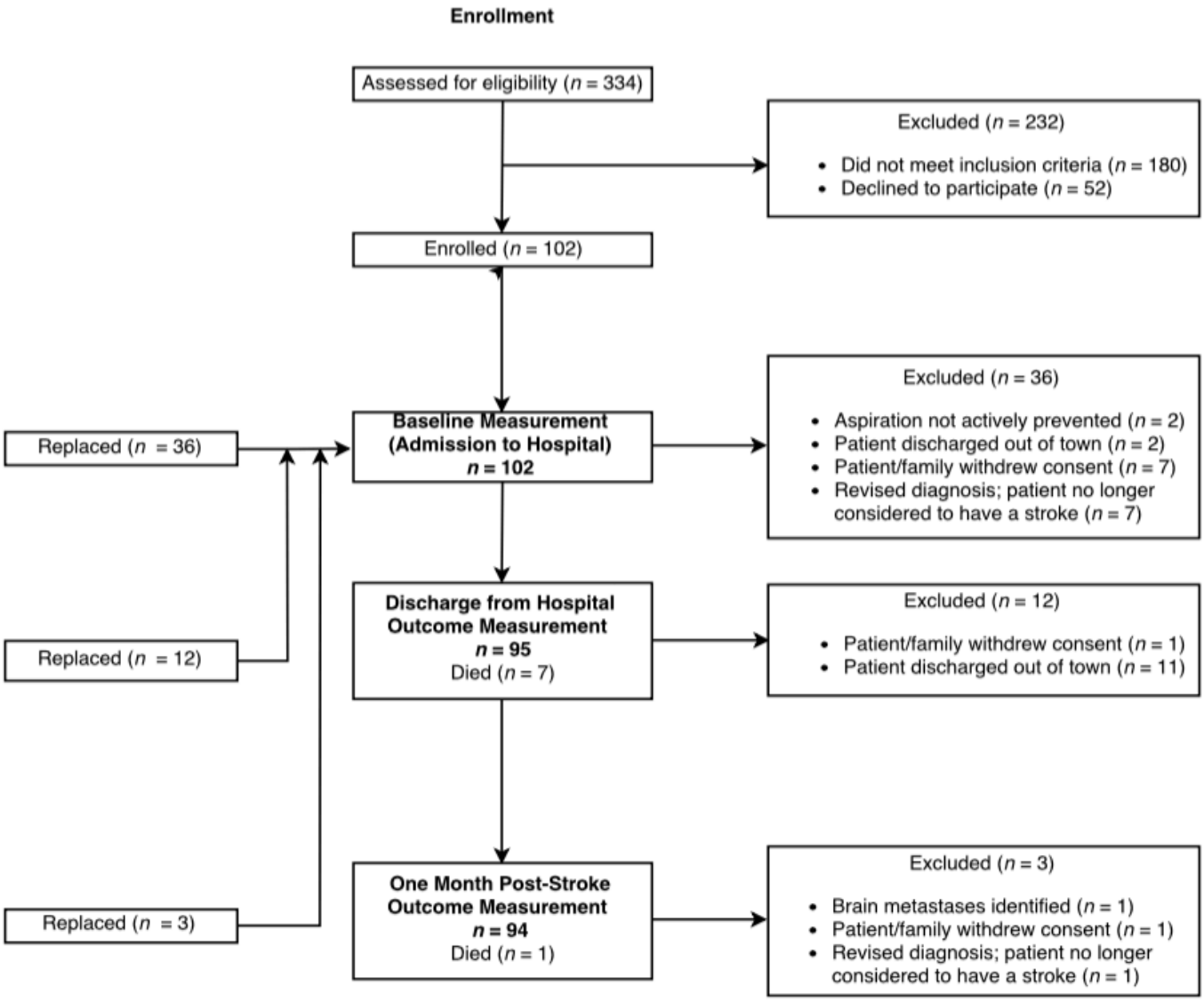


Figure 11. Details of Study Recruitment

samples were obtained at least twelve hours after the patient's last CRT. Constraints of participant availability and hospital protocols around CRT meant that time of bacterial sampling could not be controlled for, however the time of sampling was recorded with the participants' dental status (own teeth versus dentures), smoking status and dependence on others for oral care.

10.2.3.2. Cough reflex threshold measurement.

To measure cough reflex sensitivity, participants underwent CRTT (Monroe et al., 2014) using a Turboneb 2 nebuliser (Clement Clarke International Limited). Citric acid diluted in 0.9 % sodium chloride was prepared in concentrations ranging from 0.1 mol/L up to 1.2 mol/L, increasing in 0.1 mol/L increments. Solutions of citric acid were prepared weekly. CRTT was performed using a tidal breathing/face mask method whereby citric acid was delivered via a facemask (Hudson Micro Mist Nebuliser Model 41893, Standard Connector & Adult Mask) placed over the nose and mouth. The facemask was connected to a nebuliser with an obstructed flow rate of 6.6 L/minute. Initially, a placebo dose of 0.9 % sodium chloride was presented to accustom the participant to the presentation of nebulised air. Prior to presentation of citric acid participants were instructed to "breathe normally through your mouth. Try not to cough". Following this, citric acid was delivered at increasing concentrations, starting at 0.1 mol/L. Citric acid was presented for up to 15 seconds, during which time coughing presence/absence was noted. Suppressed, as opposed to natural, cough thresholds were selected for outcome measurement as this more closely represents a true reflex cough (Hegland et al., 2012).

The test was repeated up to three times at each dose, with at least 30 seconds between presentations to prevent tachyphylaxis (Morice et al., 2007). Coughing response was considered positive if two or more consecutive coughs were triggered

(C2 response threshold) on two out of three trials (Morice et al., 2007). The lowest dose of citric acid that elicited a positive response was recorded as the participant's cough reflex threshold.

CRTT were determined three times for each participant: i) upon admission to hospital, ii) at discharge from hospital and iii) at one month post-stroke. If a participant discharged on the same day as the admission test, outcome measurement data from their admission test were carried over to discharge outcome measurement data.

10.2.3.3. Aspiration pneumonia.

The third outcome of interest was the number of patients who developed AP over a one-month post-stroke period. To measure this, participants' medical charts and general practitioner records were reviewed. A diagnosis of AP was made according to previously-described criteria (Mann et al., 1999), specifically, the presence of ≥3 of the following: fever (>38°C), abnormal chest examination (tachypnea [>22 breaths/minute], tachycardia, inspiratory crackles, bronchial breathing), productive coughing with purulent sputum, abnormal chest x-ray, arterial hypoxemia (PO_2 < 70 mm Hg) and detection of a relevant pathogen (positive Gram stain and culture), as determined by a physician and/or nurse. Secondary outcome measures were also collected, including: demographic information (age, gender, ethnicity), comorbidities, length of hospital stay, oral feeding status (oral vs. non-oral), diet and place of residence at one month. Outcome measurement was conducted by the primary researcher (S.D.) via telephone or facsimile to participants, family members, residential care staff and/or general practitioners as well as a retrospective chart review at one month.

10.2.4. Data extraction.

10.2.4.1. Quantitative polymerase chain reaction assay design.

10.2.4.1.1. Quantitative polymerase chain reaction standards.

In qPCR, standards of known gDNA quantity are included as internal positive controls. By including at least one sample in each assay that is known to contain bacterial gDNA, it is possible to distinguish between true negative amplification, as opposed to unintentional qPCR inhibition, in samples of unknown gDNA quantity. Type strains *S. mitis* (ATCC 903)*, S. pneumoniae* (NZ TCC 3517)*, K. pneumoniae* (NZRM 482)*, S. aureus* (ST 92429)*, E. coli* (DH5α) and *P. aeruginosa* (OT15) were used as positive controls. Details of each specific strain are listed in Table 10.

10.2.4.1.2. Preparation of genomic DNA.

Purified genomic DNA (gDNA) were extracted from pure cultures of each type strain initially using the NucleoSpin® microbial DNA kit (Macherey-Nagal, Düren, Germany) as per the manufacturer's instructions. This method uses the mechanical force of tiny glass beads ('bead-beating') to break down the bacterial cell wall and release the gDNA. The results of this process were evaluated using spectrophotometry to measure DNA yields (NanoVue™ UV, GE Healthcare, Little Chalfont, Buckinghamshire, UK). The resulting DNA yields were unacceptably low (< 2 ng/ μL). The process was repeated for *S. mitis, K. pneumoniae, S. pneumoniae, E. coli* and *P. aeruginosa* using the PureLink Genomic DNA kit (Invitrogen™, Carlsbad, California, USA) according to the manufacturer's instructions. This method relies upon the addition of enzymes to achieve cell lysis and the release of gDNA. *S. aureus* gDNA was provided by Dr Trudy Milne (Otago University). The purified gDNA was stored at -20°C until qPCR analysis. Amplification of gDNA from bacterial isolates was by endpoint PCR followed by agarose gel electrophoresis.

Table 10

Bacterial Strains and Culture Conditions.

Bacterium	Gram-type	Strain designation	Culture conditions
S. mitis	Positive	ATCC 903	Cultured on blood agar under anaerobic then aerobic conditions. Transferred to brain-heart infusion and incubated overnight at 37°C.
P. aeruginosa	Negative	OT 15	
K. pneumoniae	Negative	NZRM 482	
S. pneumoniae	Positive	NZRM 3517	Cultured on blood agar under aerobic conditions. Transferred to brain-heart infusion and incubated overnight at 37°C.
S. aureus	Positive	NZ isolate ST 92/492	
E. coli	Negative	DH5α	

Note. ATCC = American Type Culture Collection, NZRM = New Zealand Reference Culture Collection, NZ = New Zealand, OT = Otago University Department of Microbiology and Immunology collection

10.2.4.1.3. Endpoint PCR reaction conditions.

PCR reactions were prepared in volumes ranging from 20 – 25 µL. Each reaction tube contained 10 – 12.5 µL qPCR mastermix (2x Fast SYBR® Green Master Mix, Thermo Fisher Scientific Incorporated, Waltham, Massachusetts, USA), 4.2 – 6 µL PCR-grade water (UltraPure™ DNase/RNase-free Distilled Water, Thermo Fisher), 5 µL gDNA template and 0.4 – 0.75 µL appropriate forward and reverse primers (20 µM). PCR was performed in a PTC-100 Programmable thermal controller (MJ Research, Waltham, Massachusetts, USA) using the following protocol:

1. Initial DNA denaturation and polymerase activation at 95°C for ten minutes
2. Thirty-five cycles of denaturation at 95°C for fifteen seconds, annealing (binding of primers to DNA) at 58°C for fifteen seconds and extension at 72°C for one minute
3. Final extension at 72°C for ten minutes

10.2.4.1.4. Agarose gel electrophoresis conditions.

PCR products were visualised by agarose gel electrophoresis. To make a 1.6 % gel, 1.28 g agarose (Bio-Gel A Agarose Gel, Bio-Rad Laboratories Incorporated, Hercules, California, USA) was added to 80 mL Tris buffer solution and heated in a microwave until the agarose dissolved. Once fully dissolved, 1 µL of ethidium bromide (0.5 µg/mL; Bio-Rad Laboratories) was added to the agarose which was then cooled until reaching ~55 °C. The preparation was poured into a casting tray with combs *in situ* and allowed to set for at least 20 minutes before the combs were removed. The gel was placed into a tank with approximately 300 mL Tris-acetate-EDTA (TAE) buffer (40mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3) and 0.5 µL ethidium bromide was added to the positive terminal end. DNA calibration ladder

(10 µL, 1 Kb Plus DNA Ladder, Invitrogen™, Carlsbad, California, USA) was loaded into the lateral wells of the gel. Gel loading dye (3 µL, 6X DNA Loading Dye, Thermo Fisher Scientific Incorporated, Waltham, Massachusetts, USA) was added to 10 µL of each PCR product, mixed via pipetting and 13 µL of the preparation was loaded into each well of the gel. A current was applied to the gel at a fixed voltage for 35 – 50 minutes.

The gel was removed from the tank and visualised under ultraviolet light using a Gel Doc™ EZ System (Bio-Rad Laboratories). Image Lab™ software (Bio-Rad Laboratories) was used to photograph the gel.

10.2.4.1.5. qPCR primer design.

The primer sequences were adopted from published reports and are listed in Table 11. Primers were synthesised by Sigma-Aldrich (Castle Hill, New South Wales, Australia). The Sequence Manipulation Suite: PCR products online tool (www.bioinformatics.org/sms2/pcr_products.html) was used to align the primers to their corresponding DNA sequences to determine the size of expected PCR products (listed in Table 11).

10.2.4.2. Measuring oral bacteria in saliva samples.

10.2.4.2.1. DNA extraction.

To prepare the saliva samples for analysis, the cotton tip of each swab was placed into a 15 mL Falcon™ tube (Corning Incorporated, Acton, Massachusetts, USA) containing 2 mL of phosphate-buffered saline (Gibco®, Life Technologies Corporation, Carlsbad, California, USA). Each tube was then vortexed for two 30-second bursts. The cotton tips were then discarded. Tubes were centrifuged at room temperature for three minutes at 14,000 x g to pellet the bacteria. The supernatant was aspirated and discarded and 200 µL of InstaGene™ Matrix (Bio-Rad Laboratories)

Table 11

Locations, Optimised Reaction Concentrations and Annealing Temperatures of Primers for qPCR

Target bacteria	Gene target	Sequence (5' – 3')	Tm (°C)	Expected product size (bp)	Reference
Streptococcus	16s			130	Rudney, Pan & Chen (2003)
	F	AGA TGG ACC TGC GTT GT	59.2		
	R	CT GCC TCC CGT AGG AGT CT	63.4		
S. pneumoniae	*cps*			72	Ewan, Sails, Walls, Rushton
	F	GTG TCG CTG TTT TAG CAG ATA GTG A	65.3		& Newton (2015)
	R	TCC CAG TCG GTG CTG TCA	67.2		
S. aureus	*fem*B			122	Ewan et al. (2015)
	F	GACATTTGATAGTCAACGTAAACGTA	61.8		
	R	GCTCTTCAGTTTCACGATATAAATCTAAGA	64.4		

K. pneumoniae	*pho*E			69	Shannon, Lee, Trevors &
	F	CCTGGATCTGACCCTGCAGTA	66.4		Beaudette (2007)
	R	CCGTCGCCGTTCTGTTTC	66.9		
P. aeruginosa	*ecf*X			65	Ewan et al. (2015)
	F	GCC TGT CCC AGG TCG AAG T	66.7		
	R	GAT GTG CTT TTC CAC CAT GCT	66.1		
E. coli	*uid*A			67	Ewan et al. (2015)
	F	CGC GCT TTC CCA CCA A	67.4		
	R	CGG CCT GTG GGC ATT C	66.8		

Note. Tm = melting temperature, bp = base pairs, F = forward, R = reverse

was added to each tube and mixed. The tubes were incubated in a 56 °C water bath for 30 minutes to inactivate PCR inhibitors and degradative enzymes. Following this, the tubes were vortexed for ten seconds to resuspend the InstaGene™ beads and tubes were then incubated in a 100 °C water bath for eight minutes in order to lyse the bacteria and release genomic DNA. Tubes were cooled on ice, vortexed for ten seconds and centrifuged for three minutes (14,000 x g) at room temperature to pellet the InstaGene™ beads. An aliquot (150 µL) of the supernatant containing genomic DNA was collected in 1.5 mL microcentrifuge tubes and stored at -20 °C until qPCR analysis. qPCR with a universal bacterial primer set and qPCR standards (0.2 ng/PCR well) was used to confirm the presence of target bacteria and quantify levels of gDNA extracted from twenty participant swabs prior to the final analysis. The qPCR cycling parameters are described below.

10.2.4.2.2. Quantitative polymerase chain reaction (qPCR).

To perform the qPCR assays, a preparation consisting of 0.2 µL (20 µM) of each forward and reverse primers (0.4 µL total), 4.2 µL of PCR-grade water and 10 µL of 2x Fast SYBR® Green Master Mix was dispensed into each well of a barcoded qPCR array plate (MicroAmp® EnduraPlate Optical 96-Well Fast Green Reaction Plate, Applied Biosystems, Foster City, California, USA). To duplicate wells, 5 µL of gDNA purified from each saliva sample was added. Purified gDNA from pure cultures of target bacteria (0.4 ng/µL) served as positive controls and ultra-pure water served as the negative control for each plate. Thermal cycling fluorescent measurement of double-stranded DNA was performed using the QuantStudio™ 6 Flex System.

The qPCR cycling parameters for measuring *S. aureus* and *K. pneumoniae* were: 20 seconds at 95 °C followed by 40 cycles of three seconds at 95 °C and 30

seconds at 60 °C. The PCR cycling parameters for measuring *Streptococcus, S. pneumoniae, E. coli* and *P. aeruginosa* were: 20 seconds at 95°C followed by 40 cycles of three seconds at 95 °C and 30 seconds at 63 °C. Fluorescence was measured during each cycle and the Cq calculated. The assays were calibrated with a dilution series of purified gDNA from each of the target bacteria. Linear amplification was assessed over a range of 0.002 – 2 ng/well (*S. aureus*), 0.002 – 0.11 ng/well (*S. mitis*), 0.0002 – 2 ng/well (*K. pneumoniae*), 0.04 – 0.4 ng/well (*S. pneumoniae*), 0.0004 – 0.4 ng/well (*P. aeruginosa, E. coli*).

The amplification efficiency of each assay was calculated using the formula (Rasmussen, 2001):

$$\text{Efficiency} = \left[10^{\left(\frac{-1}{\text{slope}}\right)} - 1\right] \times 100\ \%$$

10.2.5. Data analysis.

Statistical analyses were undertaken with SPSS (IBM Corp.). Using a 2x2 Chi-square test based on the proportion of patients with AP colonised with *S. mitis* and *S. pneumoniae* (Bousbia et al., 2012), an *a priori* sample size of 102 participants was calculated for an estimated effect size of 0.4 and 90 % statistical power ($p < .05$).

For statistical analysis, the raw data from the qPCR was transformed from quantitation cycles to relative numbers of bacteria using the formula:

$$\text{Relative number of bacteria} = 2^{-Cq}$$

The relative number of bacteria is a unitless measure and in this context refers to the number of bacteria in one sample relative to the next.

Certain bacteria (i.e. *Streptococcus*) are commonly found in the oral cavities of normal, healthy people and were expected to be present in most of the samples. For this reason, it was decided to categorise the relative levels of bacteria as ‘high’, ‘medium’ or ‘low’ for regression analysis.

Linear mixed modelling was used to measure changes in the levels of each bacterial species over time. A one-way repeated-measures analysis of variance (ANOVA) was performed to determine changes in total bacteria levels over time, controlling for age, gender, days spent in hospital, dependence for oral cares, smoking status, time of testing and oral feeding status. Participants who died during hospitalisation were excluded from this analysis (n = 10). Because fourteen participants discharged within 24 hours of baseline testing, the same data were used for their discharge measures. Repeat linear mixed modelling as well as a second one-way repeated-measures ANOVA were run to determine if excluding these participants from the dataset made a difference to the ability to measure changes in bacterial levels over time. Logistic regressions were used to predict bacterial levels (high, medium, low) at i) admission to hospital, ii) discharge from hospital, iii) one month post-stroke, adjusting for gender, age, smoking status, diabetes mellitus, dentition, ability to complete oral care independently, time of testing and number of days in hospital (for discharge and one-month measures only). Logistic regressions were also used to predict levels of specific target bacteria (high, medium, low), adjusted for age, gender and dependence for oral care.

A one-way repeated-measures ANOVA was performed to determine changes in cough reflex thresholds over time, controlling for time of testing, age, gender and smoking status. Again, fourteen participants discharged within 24 hours of baseline testing, therefore the same data were used for their discharge measures. Multiple linear regressions were used to predict cough reflex thresholds at 1) admission, 2) discharge, 3) one month post-stroke, adjusted for gender, age and smoking (block one) and levels of *Streptococcus, S. pneumoniae, S. aureus, E. coli, K. pneumoniae* and *P. aeruginosa* and time of testing (block two).

Multinomial logistic regression was used to predict the occurrence of aspiration pneumonia at admission to hospital, discharge from hospital and at one month post-stroke, adjusted for the following variables: age, gender, cough reflex sensitivity, total bacteria levels at admission, discharge and one month and being in hospital for more than 30 days. A second model that included levels of *Streptococcus, S. pneumoniae, S. aureus, K. pneumoniae, E. coli* and *P. aeruginosa* at admission, discharge and one month was also run.

Diet tolerance was categorised as i) tube-fed with no/minimal oral intake, ii) modified oral diet +/- supplemental tube feeding, or iii) normal diet. Participants' ability to perform oral care independently was treated as a binary variable. Participants were considered to be smokers if they reported smoking within six weeks prior to admission to hospital. Dentition was categorised as: own teeth, own teeth plus dentures, full dentures or edentate. Time of testing was categorised as morning (8am – 11.59am), noon (12pm – 2pm) or afternoon (2.01pm – 5pm).

10.3. Results i) Preparatory Analyses

This section contains the results of preliminary analyses that were required to determine the sensitivity and specificity of the qPCR assay. This series of experiments was completed prior to the analysis of patient samples.

10.3.1. Analysis of genomic DNA.

The purity and concentration of gDNA extracted from each of the pure cultures is listed in Table 12. A_{260}:A_{280} ratios of 1.8 – 2.0 indicate appropriate DNA purity (Desjardins & Conklin, 2010). Endpoint PCR and agarose gel electrophoresis

Table 12.

Comparison of Bacterial gDNA Purity and Yield by Two Lysis Methods

gDNA template	Mechanical lysis[1]		Enzymatic lysis[2]	
	A_{260}:A_{280}	Concentration (ng/μL)	A_{260}:A_{280}	Concentration (ng/μL)
S. mitis	2.00	1.76	1.90	7.72
S. pneumoniae	2.25	0.18	2.04	4.56
K. pneumoniae	2.00	0.40	1.72	1.10
S. aureus	1.90	0.76	1.90	20.00
E. coli	1.80	0.72	1.31	8.42
P. aeruginosa	1.86	1.60	1.43	8.66

[1] bead-beating essentials (NucleoSpin® microbial DNA kit), [2] enzymatic essentials (PureLink Genomic DNA kit)

confirmed detection of identified bacterial species (Figures 12, 13, 14, 15) prior to primer optimisation for qPCR.

Assessment of the purity and concentration of the DNA extracted from the saliva samples was attempted originally spectrophotometrically. However, this was abandoned due to wide variability in repeated readings. The most likely explanation for this variability was that the concentration and/or purity of the extracted DNA was too low to be measured accurately by this method. Therefore, qPCR using a universal bacterial primer set and qPCR standards (0.2 ng/PCR well) was used to confirm the presence of target bacteria and to quantify relative levels of gDNA extracted from twenty participant samples prior to the final analysis. Cq values in the participant subset were between 24 – 37 (Figure 16). Visual inspection of melting curves confirmed the detection of target bacteria. Analysing a subset of qPCR products by gel electrophoresis also verified this (Figure 17).

A comparison of gDNA extraction method efficiency was undertaken by PCR analysis of gDNA templates that were extracted by the mechanical lysis method and by the enzymatic lysis method. The PCR products were resolved by gel electrophoresis.

10.3.2. Sensitivity of qPCR primers.

Sensitivity of the primers was assessed by preparing serial dilutions of gDNA templates. Endpoint PCR and agarose gel electrophoresis were used to compare the expected product size of each bacterial product against a 1 Kb Plus DNA calibration ladder (Figures 18, 19).

10.3.3. Specificity of qPCR primers.

Primer specificity was confirmed using the basic alignment search tool (BLAST)

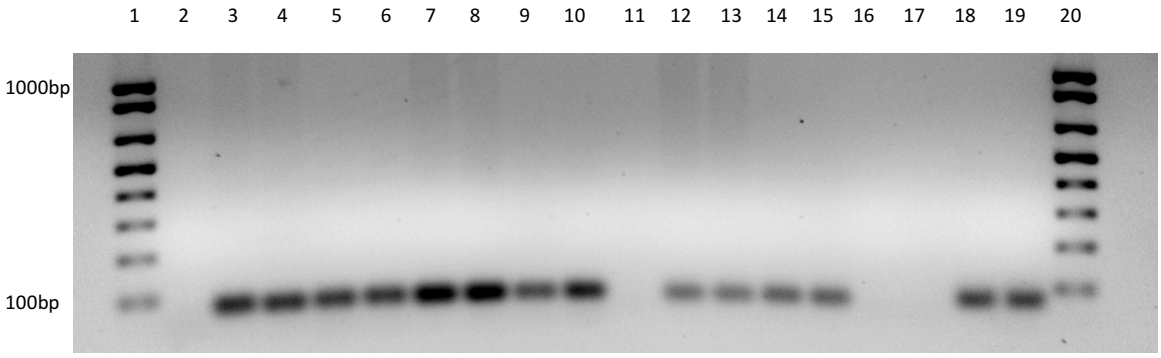


Figure 12. Validation of gDNA extraction methods, primers and PCR parameters for amplifying *K. pneumoniae* and *E. coli* by end-point PCR and agarose gel electrophoresis. Lanes 1, 20: 1 Kb plus markers; Lane 2, 11: blank; Lanes 3 – 6: *K. pneumoniae* gDNA extracted using the mechanical lysis method: 5 ng/PCR tube (Lanes 3, 4) and 0.5 ng/PCR tube (Lanes 5, 6); Lanes 7 – 10: *K. pneumoniae* gDNA extracted using the enzymatic lysis method: 5 ng/PCR tube (Lanes 7, 8) and 0.5 ng/PCR tube (Lanes 9, 10); Lanes 12-15: *E. coli* gDNA extracted using the mechanical lysis method: 5 ng/PCR tube (Lanes 12, 13) and 0.5 ng/PCR tube (Lanes 14, 15); Lanes 16 – 19: *E. coli* gDNA extracted using the enzymatic lysis method: 5 ng/PCR tube (Lanes 16, 17) and 0.5 ng/PCR tube (Lanes 18, 19). Amplified products (amplicons) were not evident in two PCR wells (lanes 16, 17). A ~100-base pair product indicates amplification of the bacterial gene targets.

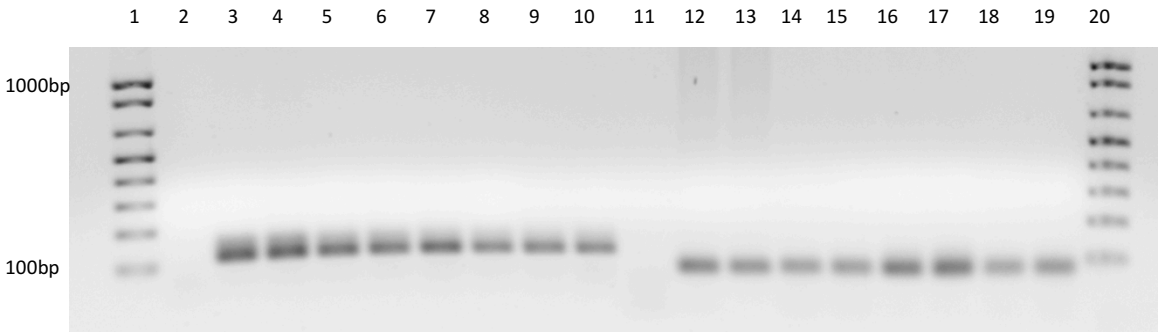


Figure 13. Validation of gDNA extraction methods, primers and PCR parameters for amplifying *S. aureus* and *P. aeruginosa* by end-point PCR and agarose gel electrophoresis. Lanes 1, 20: 1 Kb plus markers; Lane 2, 11: blank; Lanes 3 – 6: *S. aureus* gDNA extracted using the mechanical lysis method: 5 ng/PCR tube (Lanes 3, 4) and 0.5 ng/PCR tube (Lanes 5, 6); Lanes 7 – 10: *S. aureus* gDNA extracted using the enzymatic lysis method: 5 ng/PCR tube (Lanes 7, 8) and 0.5 ng/PCR tube (Lanes 9, 10); Lanes 12-15: *P. aeruginosa* gDNA extracted using the mechanical lysis method: 5 ng/PCR tube (Lanes 12, 13) and 0.5 ng/PCR tube (Lanes 14, 15); Lanes 16 – 19: *P. aeruginosa* gDNA extracted using the enzymatic lysis method: 5 ng/PCR tube (Lanes 16, 17) and 0.5 ng/PCR tube (Lanes 18, 19). A ~100-base pair product indicates amplification of the bacterial gene targets.

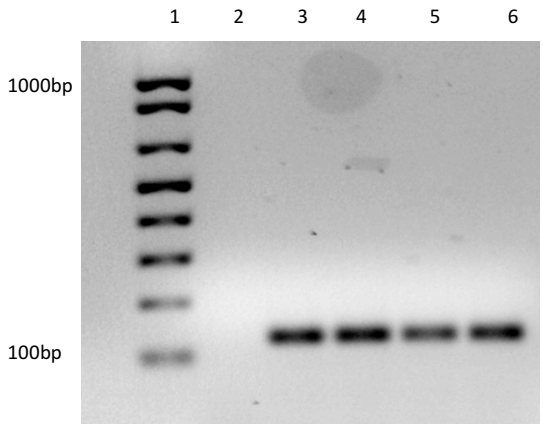


Figure 14. Validation of gDNA extraction methods, primers and PCR parameters for amplifying *Streptococcus* by end-point PCR and agarose gel electrophoresis. Visualisation of amplification products was only undertaken for *S. mitis* gDNA extracted using the mechanical lysis method. Lane 1: 1 Kb plus markers; Lane 2: blank; Lanes 3 – 6: *S. mitis* gDNA: 5 ng/PCR tube (Lanes 3, 4) and 0.5 ng/PCR tube (Lanes 5, 6). A ~100-base pair product indicates amplification of the bacterial gene targets.

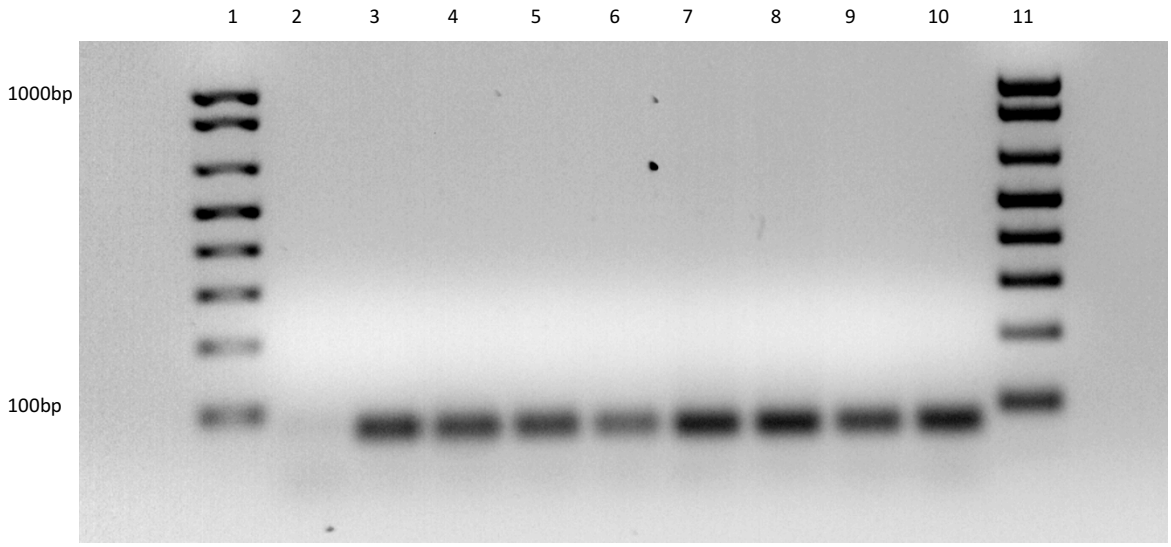


Figure 15. Validation of gDNA extraction methods, primers and PCR parameters for amplifying *S. pneumoniae* by end-point PCR and agarose gel electrophoresis. Lanes 1, 11: 1 Kb plus markers; Lane 2: blank; Lanes 3 – 6: *S. pneumoniae* gDNA extracted using the mechanical lysis method: 5 ng/PCR tube (Lanes 3, 4) and 0.5 ng/PCR tube (Lanes 5, 6); Lanes 7 – 10: *S. pneumoniae* gDNA extracted using the enzymatic lysis method: 5 ng/PCR tube (Lanes 7, 8) and 0.5 ng/PCR tube (Lanes 9, 10). A ~100-base pair product indicates amplification of the bacterial gene targets.

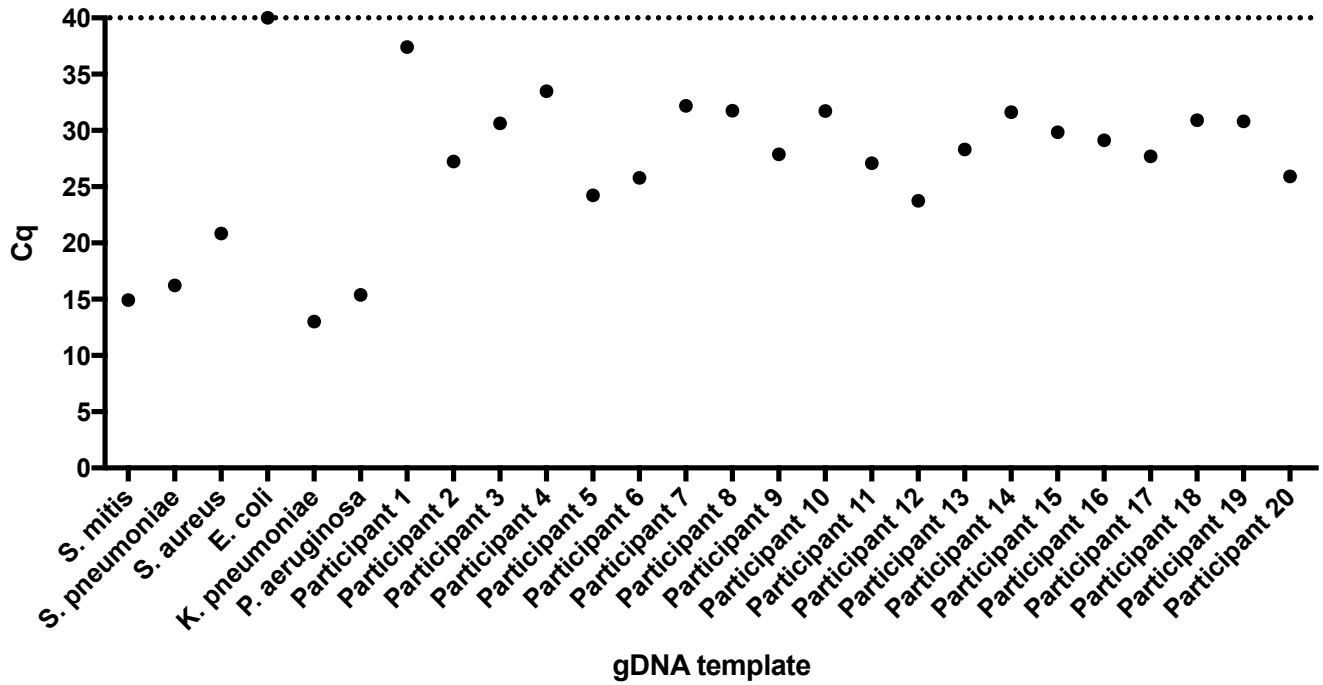


Figure 16. Relative bacterial levels in qPCR standards and gDNA samples from participants 1 – 20 using universal bacterial primers. *E. coli* was not detected. The dashed line represents the detection limit of the assay. Cq = quantitation cycle.

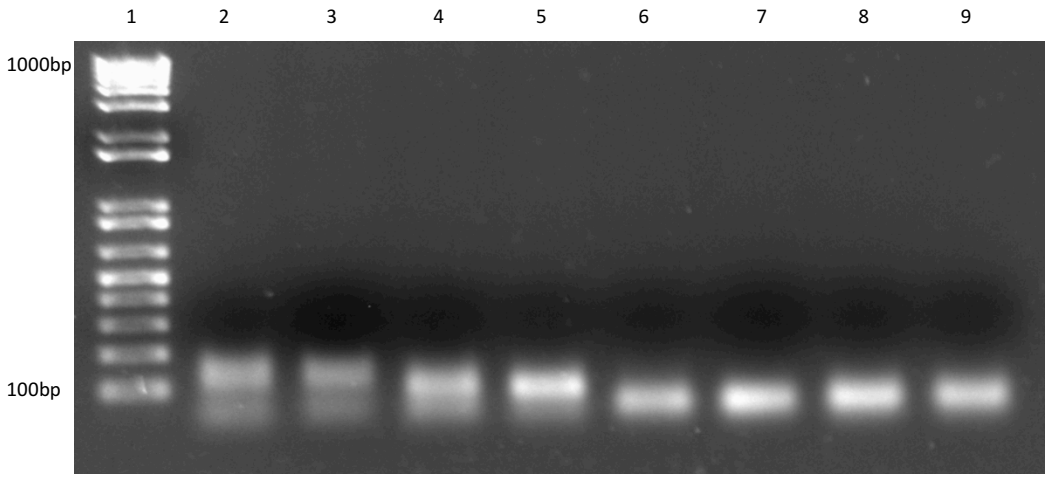


Figure 17. Example of melting curve analysis of qPCR products by agarose gel electrophoresis. Lane 1: 1 Kb Plus markers; Lanes 2, 3: qPCR products generated from *S. pneumoniae* gDNA (0.2 ng/PCR well); Lanes 4, 5: participant sample with a melting curve identical to the *S. pneumoniae* standard; Lanes 6, 7: participant sample with a melting curve that did not match the *S. pneumoniae* standard; Lanes 8, 9: water with a melting curve that did not match the *S. pneumoniae* standard. Samples were assayed in duplicate.

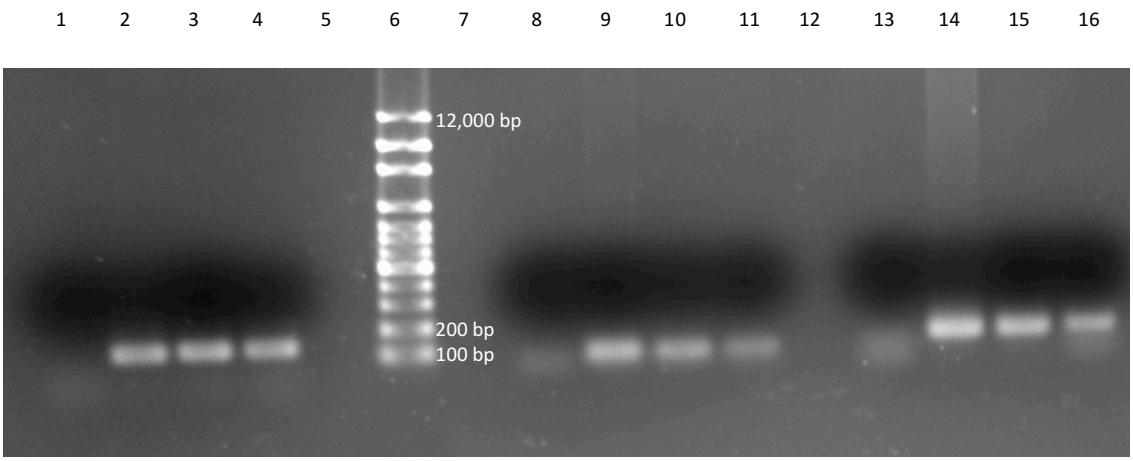


Figure 18. Validation of *Streptococcus, S. pneumoniae* and *S. aureus* primers using end-point PCR products and agarose gel electrophoresis. Expected product sizes were 130 bp (*S. mitis*), 72 bp (*S. pneumoniae*), 122 bp (*S. aureus*). Lanes 1, 8, 13: water; Lanes 2-4: serial dilutions of *S. mitis* gDNA; Lane 6: 1 Kb Plus markers (Invitrogen™); Lanes 9-11: serial dilutions of *S. pneumoniae* gDNA; Lanes 14-16: serial dilutions of *S. aureus* gDNA.

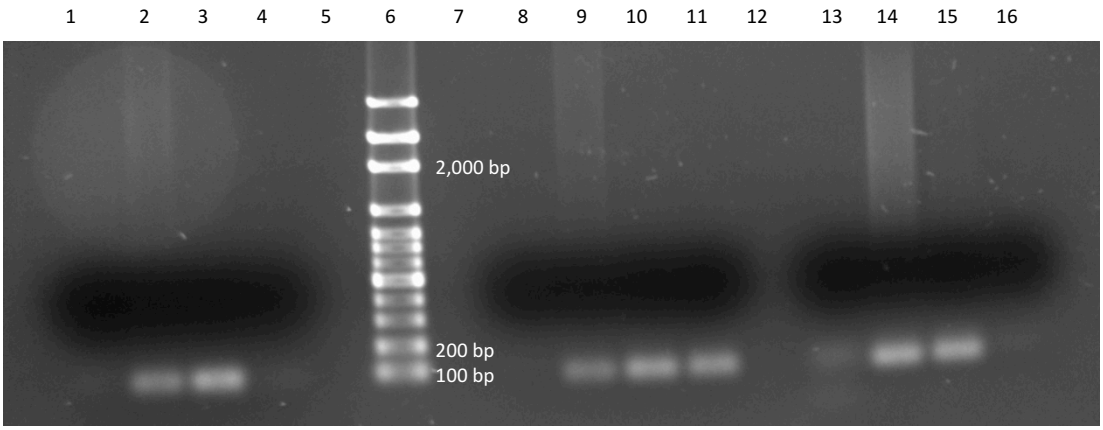


Figure 19. Validation of *K. pneumoniae, E. coli* and *P. aeruginosa* primers using end-point PCR products and agarose gel electrophoresis. Expected product sizes were 69 bp (*K. pneumoniae*), 67 bp (*E. coli*), 65 bp (*P. aeruginosa*). Lanes 1, 8, 13: water; Lanes 2-4: serial dilutions of *K. pneumoniae* gDNA; Lane 6: 1 Kb Plus markers (Invitrogen™); Lanes 9-11: serial dilutions of *E. coli* gDNA; Lanes 14-16: serial dilutions of *P. aeruginosa* gDNA.

against the GenBank nucleotide database. None of the primer sets produced unexpected matches. Specificity was further confirmed by qPCR and agarose gel electrophoresis by comparing amplicons from mis-matched primer-gDNA combinations. For each gDNA target, six PCR tubes were prepared, containing qPCR mastermix, PCR-grade water and gDNA template. One set (forward and reverse) of each primer was added to the reaction tubes such that one of six tubes received the correct primer set and five received a primer mis-match.

The resulting amplification plots are displayed in Figures 20, 23, 26, 29, 32 and 35. Ideally, the correct primer-target combination should result in a Cq value in the range of fifteen to twenty cycles, with the primer mismatches resulting in a Cq value of 40 cycles (i.e. no amplification occurred after 40 cycles). Visual inspection of melting curves also confirmed primer specificity. Figures 21, 24, 27, 30, 33 and 36 are examples of melting curves where the amplified products matched the target product (melting curves overlap) and Figures 22, 25, 28, 31, 34 and 37 are examples of melting curves where the amplified products did not match the target product (melting curves do not overlap).

As a final check of primer specificity, agarose gel electrophoresis was used to compare the product size against the 1 Kb Plus DNA ladder. Ideally, the correct primer-target combination should result in an intense band of the expected size and the primer mismatches should result in no bands. The PCR products were resolved by agarose gel electrophoresis (Figures 38 - 43).

Inspection of the amplification plots, melting curves and agarose gels indicated acceptable specificity of the *S. aureus* (Figures 26, 28, 40) and *K. pneumoniae* primers (Figures 32, 34, 42). To optimise primer annealing for the

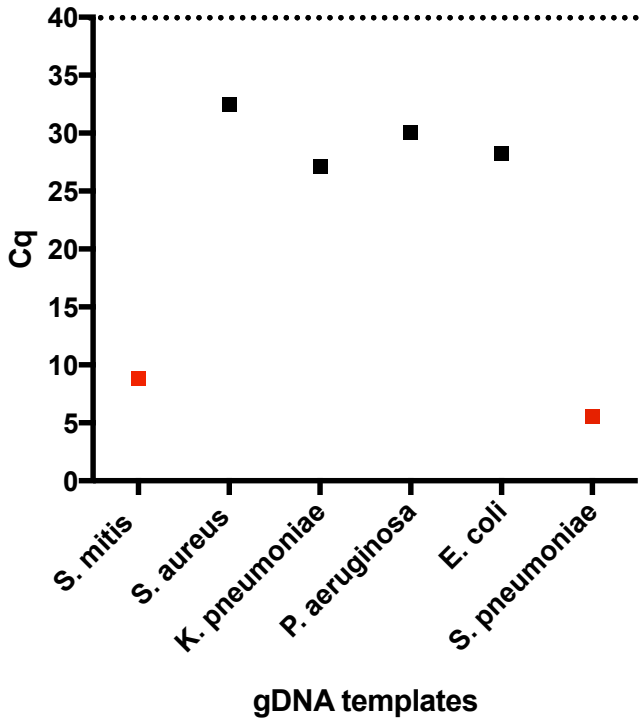


Figure 20. Mismatch assessment of *Streptococcus* primers against six bacterial standards by qPCR with an annealing temperature of 60°C. gDNA templates expected to be amplified are highlighted in red. The dashed line represents the detection limit of the assay.

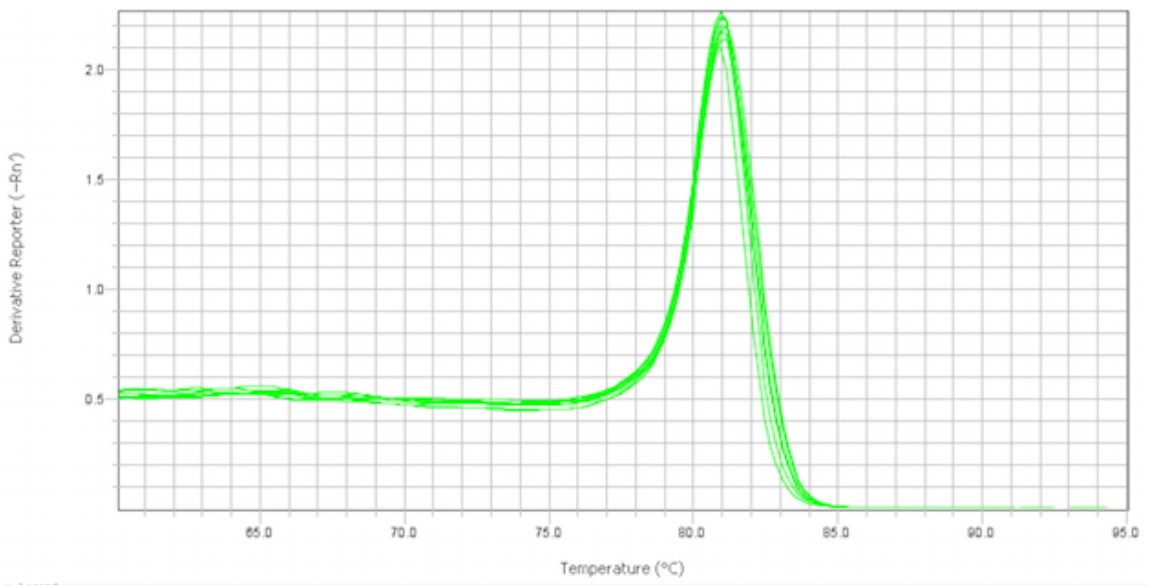


Figure 21. Melting curve of *Streptococcus* gDNA qPCR products using an annealing temperature of 60°C.

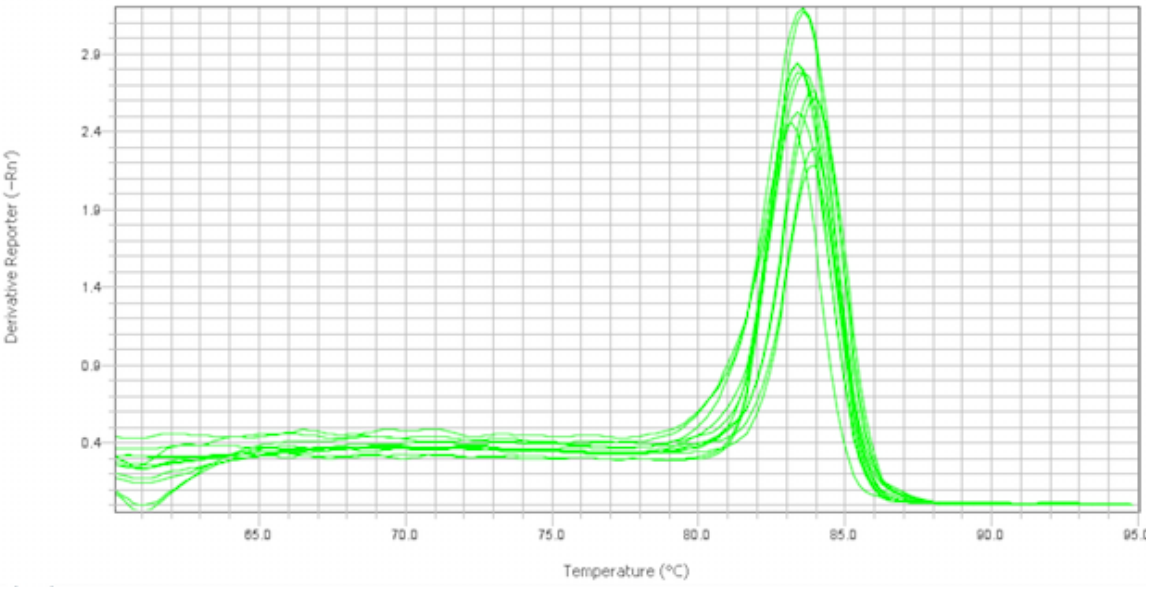


Figure 22. Comparison of melting curves of amplified qPCR products from six bacterial gDNA templates when a *Streptococcus* primer set was tested under an annealing temperature of 60 °C

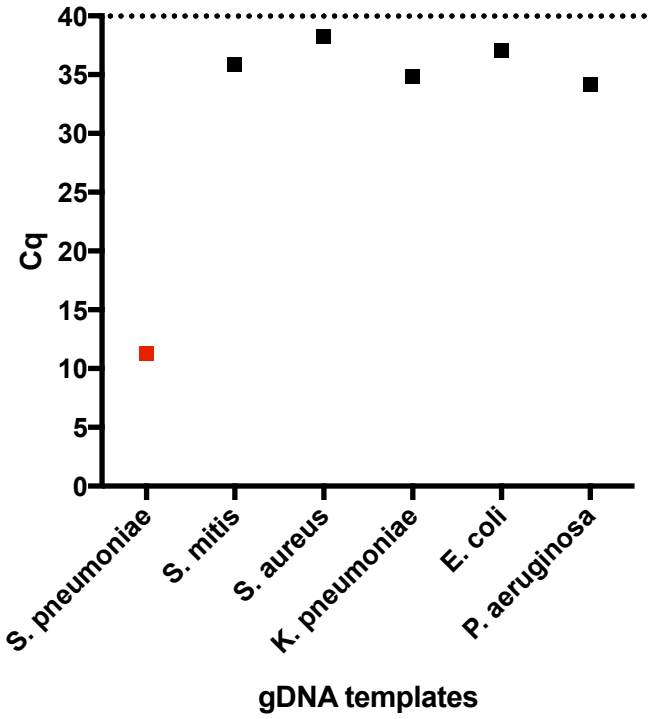


Figure 23. Mismatch assessment of *S. pneumoniae* primers against six bacterial standards by qPCR with an annealing temperature of 60°C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detection limit of the assay.

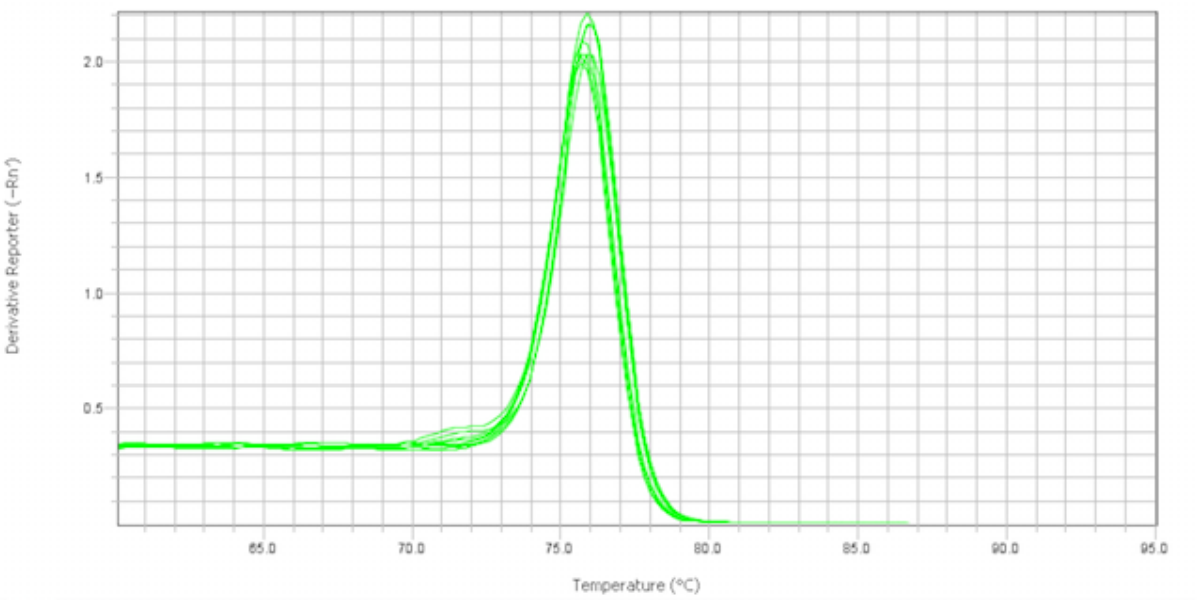


Figure 24. Melting curve of *S. pneumoniae* gDNA qPCR products using an annealing temperature of 60°C.

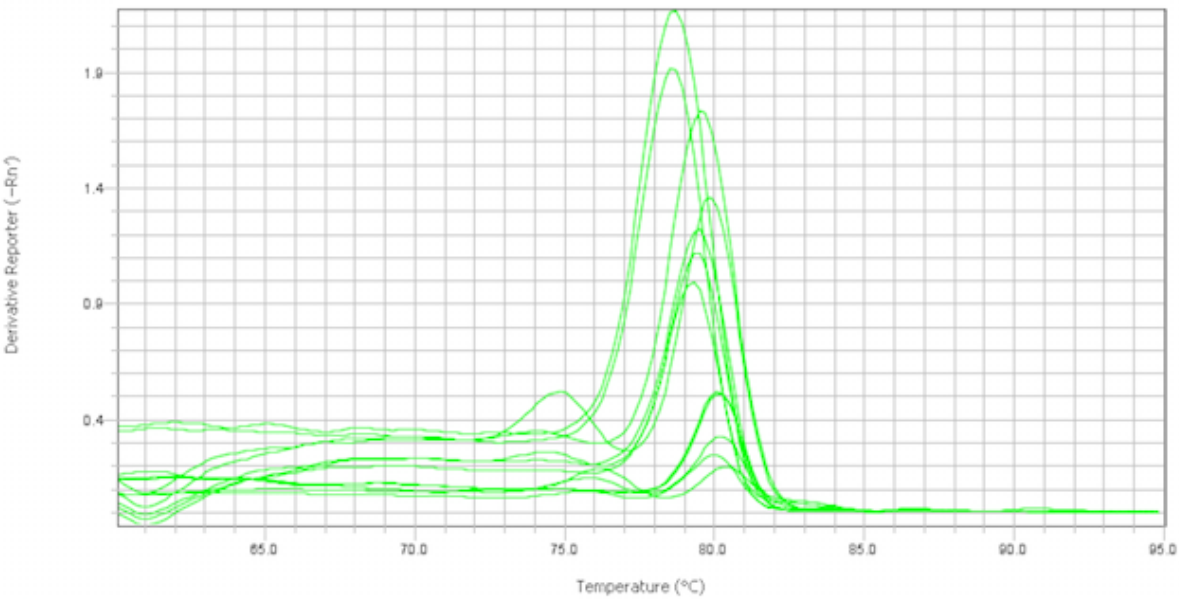


Figure 25. Comparison of melting curves of amplified qPCR products from six bacterial gDNA templates when *S. pneumoniae* primers were tested under an annealing temperature of 60 °C.

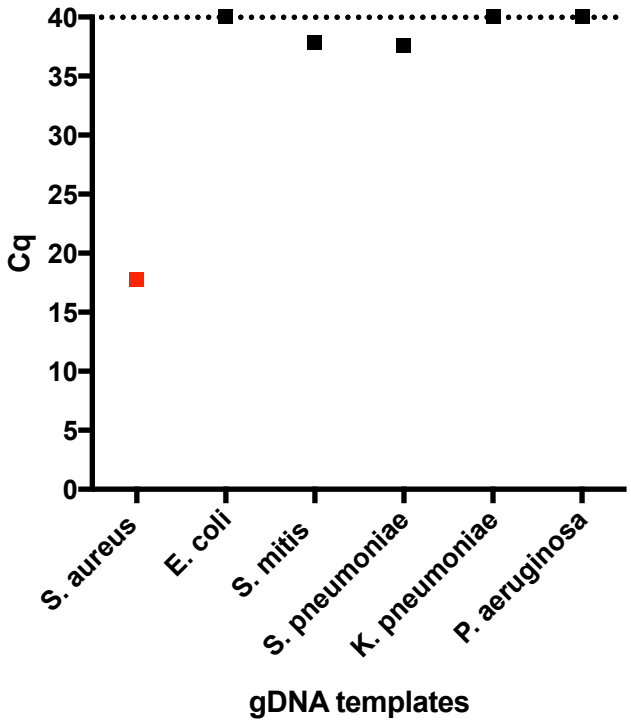


Figure 26. Mismatch assessment of *S. aureus* primers against six bacterial standards by qPCR with an annealing temperature of 60°C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detection limit of the assay.

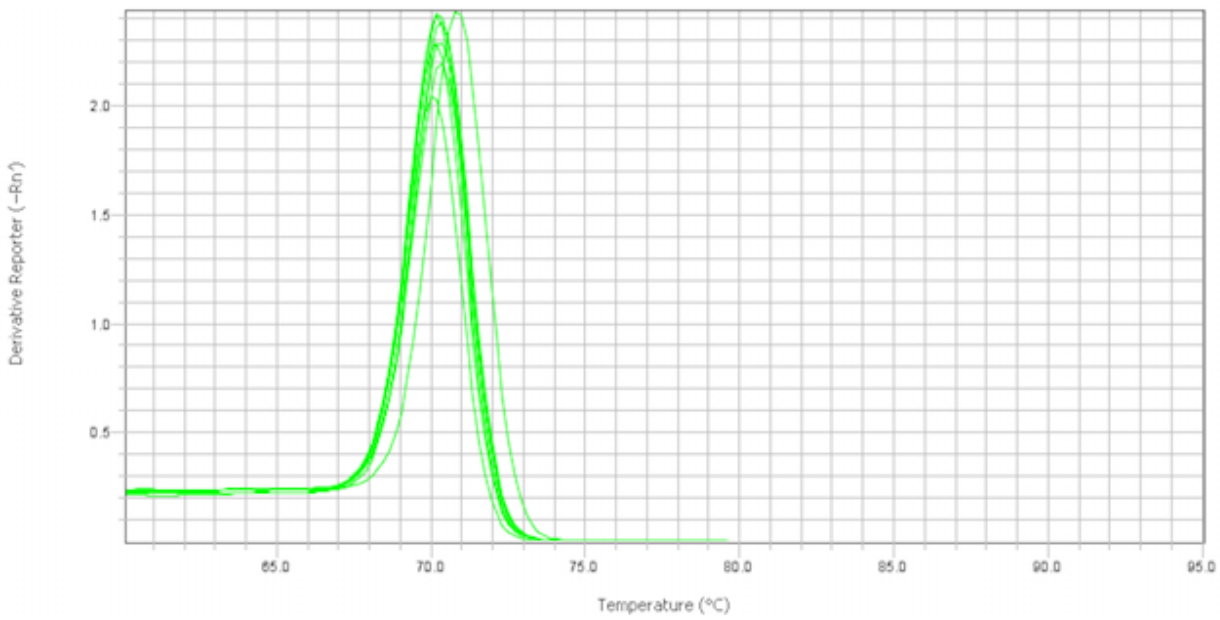


Figure 27. Melting curve of *S. aureus* gDNA qPCR products using an annealing temperature of 60°C.

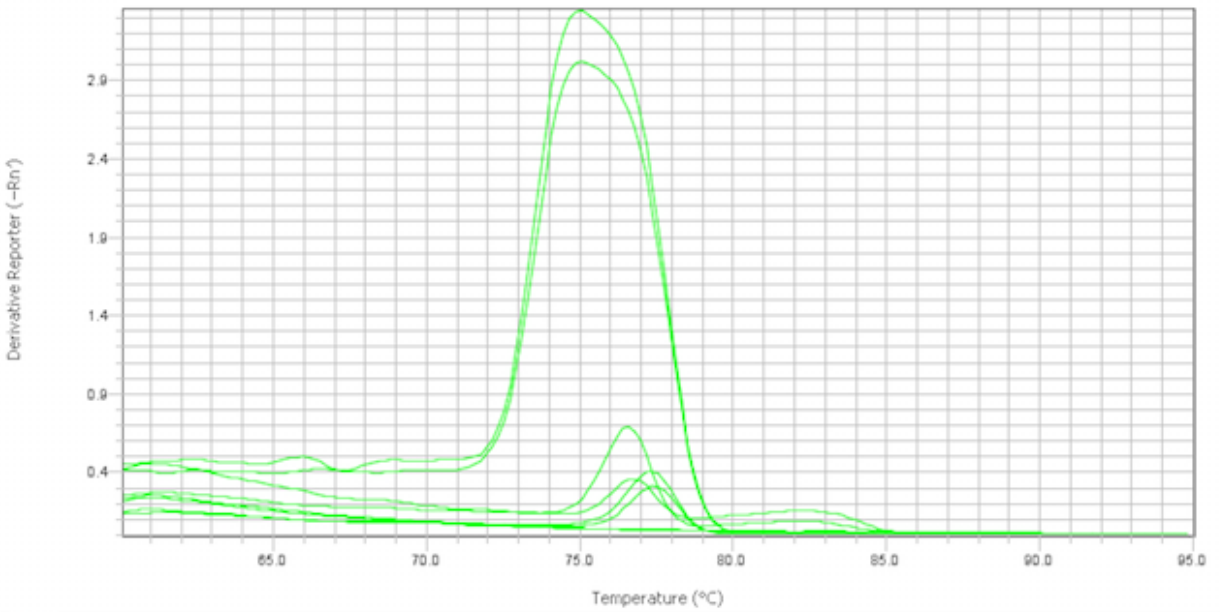


Figure 28. Comparison of melting curves of amplified qPCR products from six bacterial gDNA templates when *S. aureus* primers were tested under an annealing temperature of 60 °C.

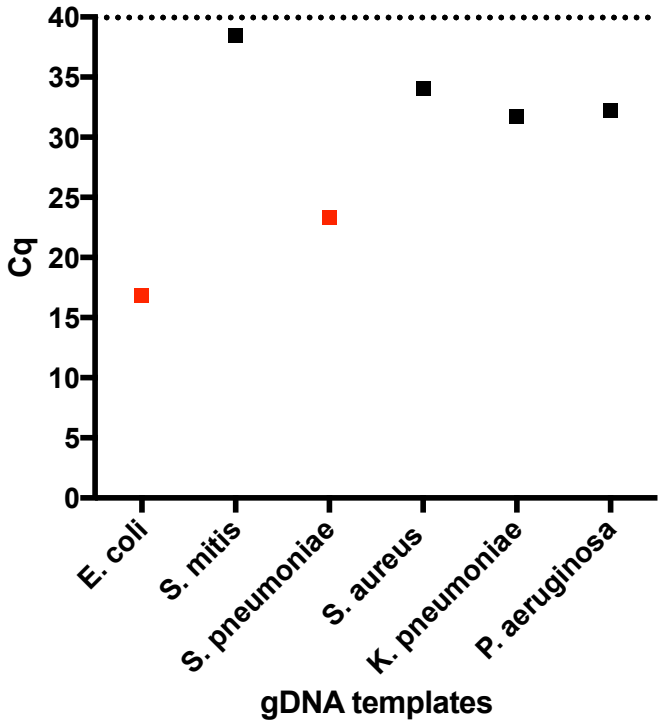


Figure 29. Mismatch assessment of *E. coli* primers against six bacterial standards by qPCR with an annealing temperature of 60°C. Efficiently amplified gDNA templates are highlighted in red. The dashed line represents the detection limit of the assay.

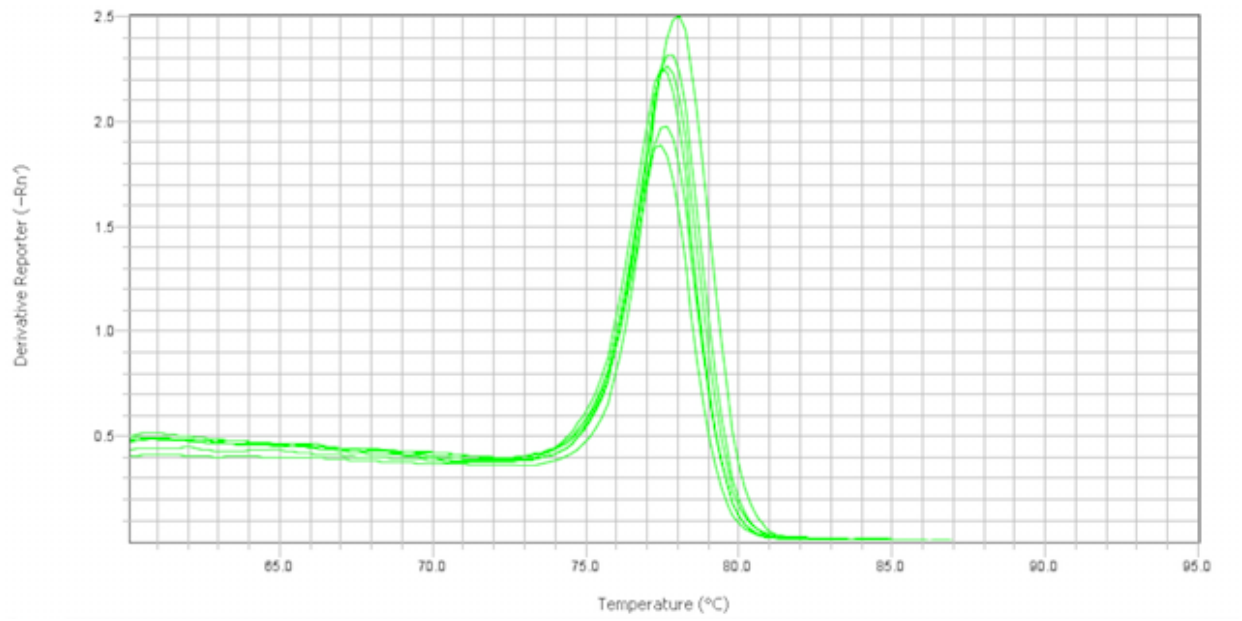


Figure 30. Melting curve of *E. coli* gDNA qPCR products using an annealing temperature of 60°C.

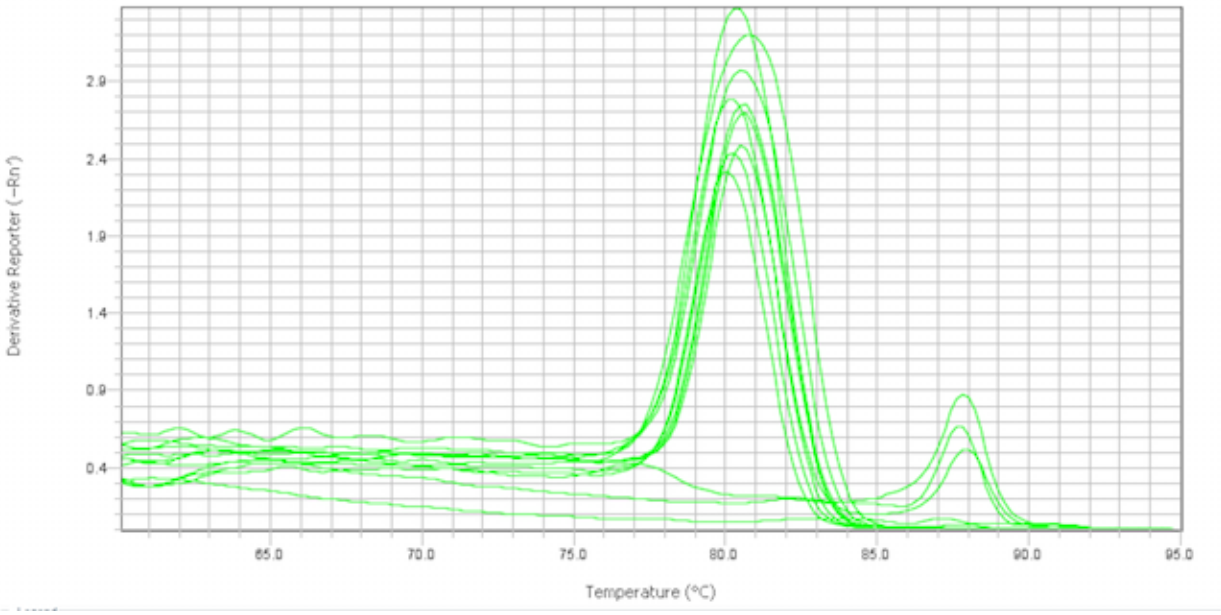


Figure 31. Comparison of melting curves of amplified qPCR products from six bacterial gDNA templates when *E. coli* primers were tested under an annealing temperature of 60 °C.

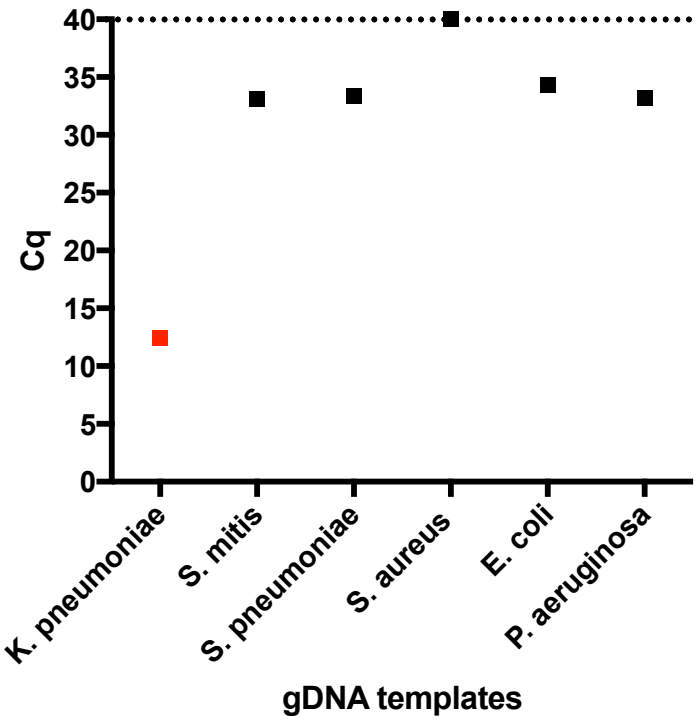


Figure 32. Mismatch assessment of *K. pneumoniae* primers against six bacterial standards by qPCR with an annealing temperature of 60°C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detection limit of the assay.

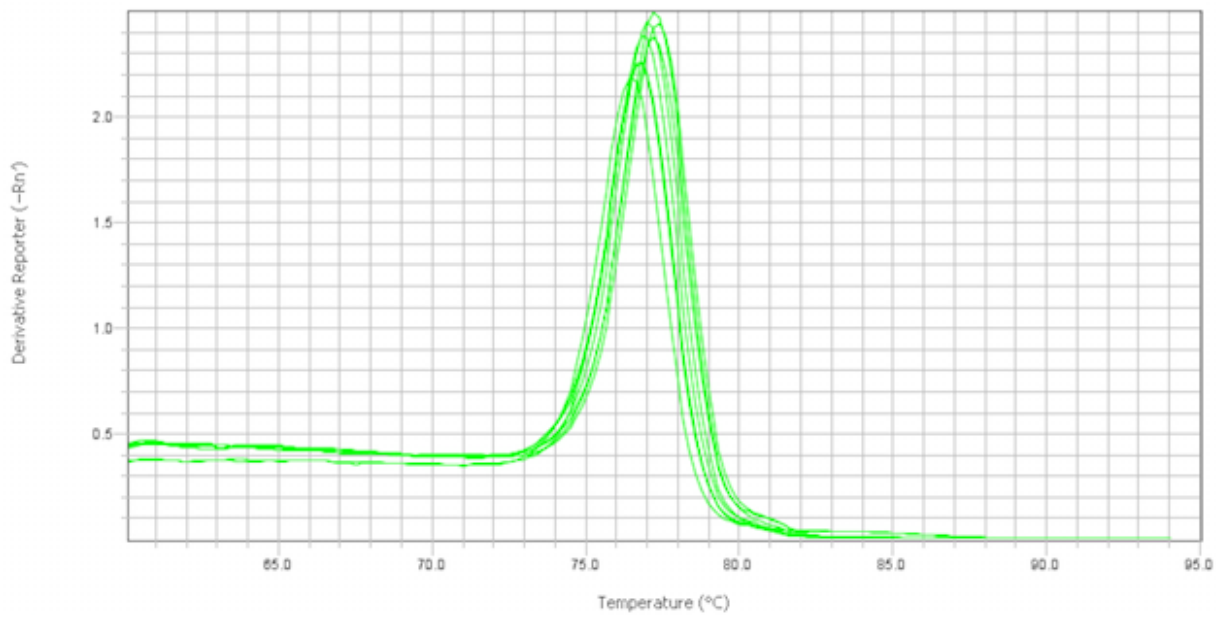


Figure 33. Melting curve of *K. pneumoniae* gDNA qPCR products using an annealing temperature of 60°C.

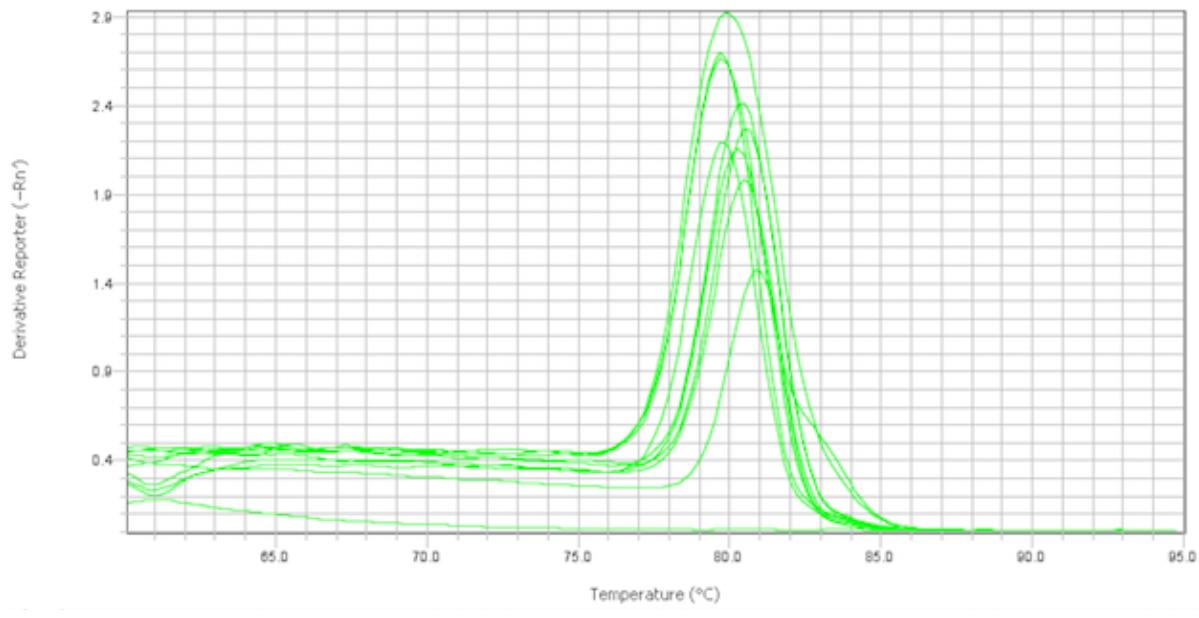


Figure 34. Comparison of melting curves of amplified qPCR products from six bacterial gDNA templates when *K. pneumoniae* primers were tested under an annealing temperature of 60 °C.

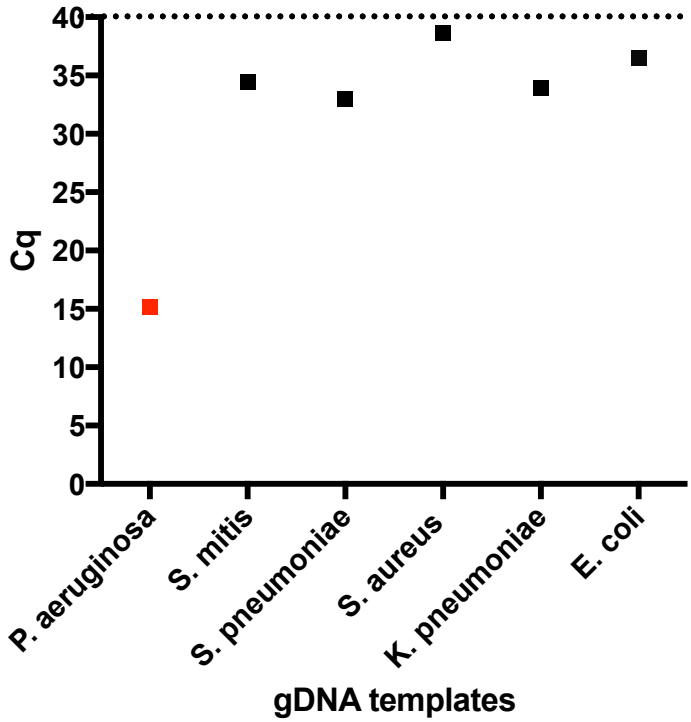


Figure 35. Mismatch assessment of *P. aeruginosa* primers against six bacterial standards by qPCR with an annealing temperature of 60°C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detection limit of the assay.

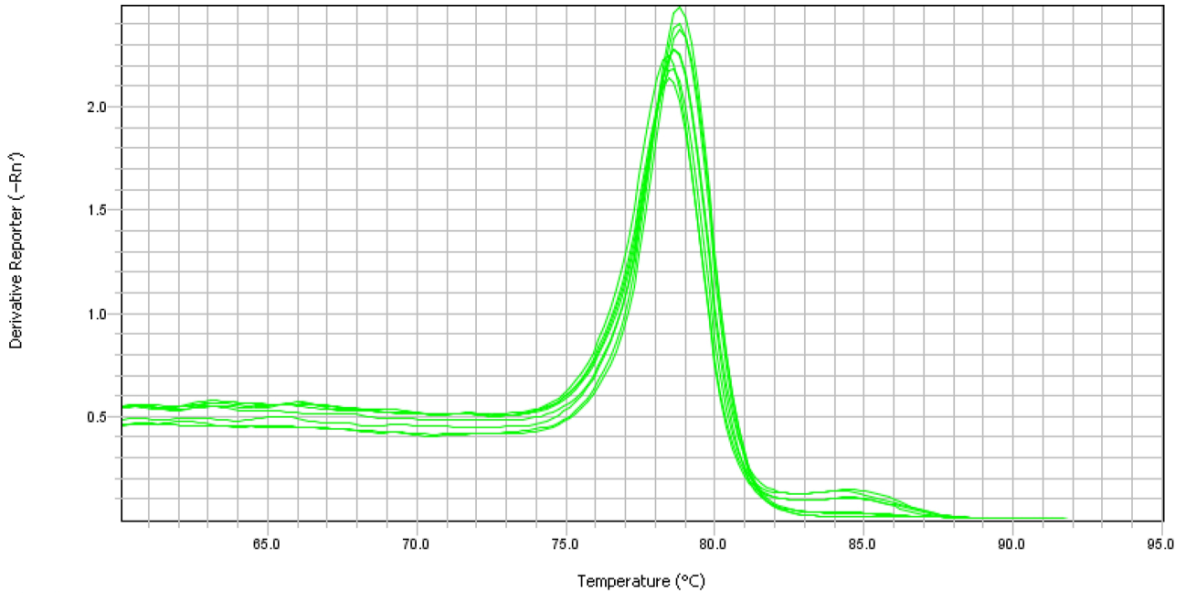


Figure 36. Melting curve of *P. aeruginosa* gDNA qPCR products using an annealing temperature of 60°C.

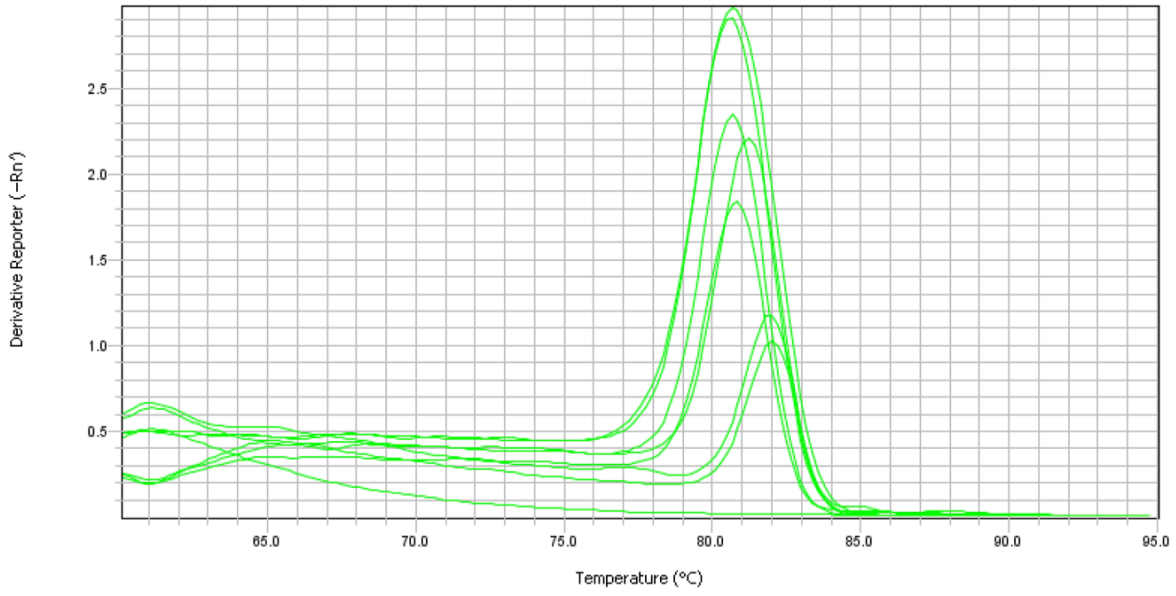


Figure 37. Comparison of melting curves of amplified qPCR products from six bacterial gDNA templates when *P. aeruginosa* primers were tested under an annealing temperature of 60 °C

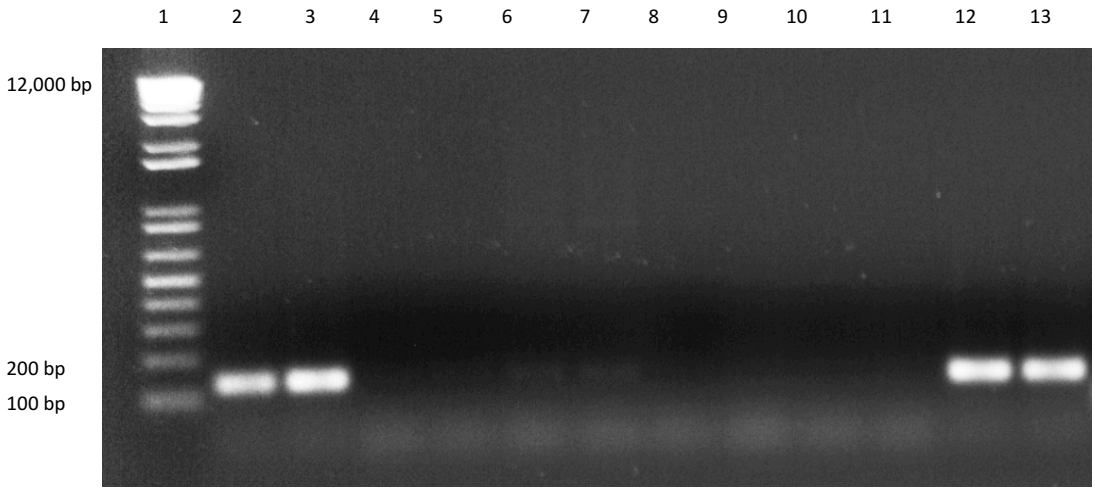


Figure 38. Validation of a *Streptococcus* primer set by agarose gel electrophoresis following qPCR with an annealing temperature of 60°C. Lane 1: 1 Kb Plus markers; Lanes 2, 3: *S. mitis* gDNA; Lanes 4, 5: *S. aureus* gDNA; Lanes 6, 7: *K. pneumoniae* gDNA; Lanes 8,9: *P. aeruginosa* gDNA; Lanes 10, 11: *E. coli* gDNA; Lanes 12, 12: *S. pneumoniae* gDNA.

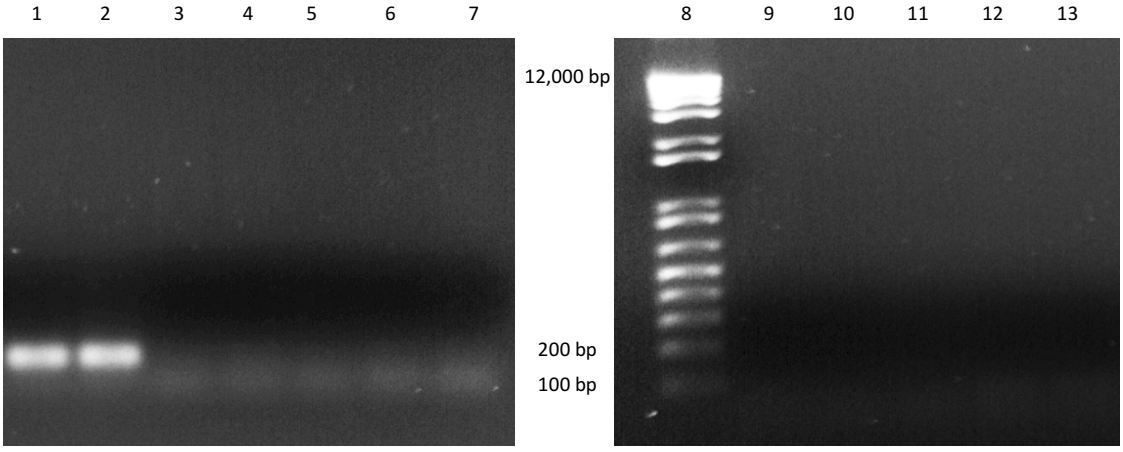


Figure 39. Validation of *S. pneumoniae* primers by agarose gel electrophoresis following qPCR with an annealing temperature of 60°C. Lanes 1, 2: *S. pneumoniae* gDNA; Lanes 3, 4: *S. mitis* gDNA; Lanes 5, 6: *S. aureus* gDNA; Lanes 7, 9: *K. pneumoniae* gDNA; Lane 8: 1 Kb Plus markers; Lanes 10, 11: *E. coli* gDNA; Lanes 12, 13: *P. aeruginosa* gDNA.

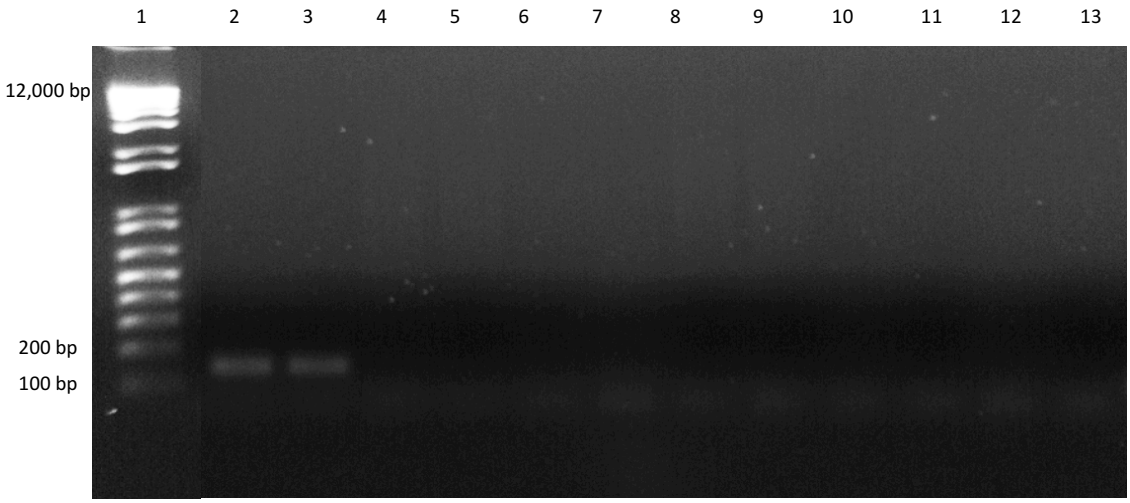


Figure 40. Validation of *S. aureus* primers by agarose gel electrophoresis following qPCR with an annealing temperature of 60°C. Lane 1: 1 Kb Plus markers; Lanes 2, 3: *S. aureus* gDNA; Lanes 4, 5: *S. mitis* gDNA; Lanes 6, 7: *S. pneumoniae* gDNA; Lanes 8,9: *K. pneumoniae* gDNA; Lanes 10, 11: *E. coli* gDNA; Lanes 12, 13: *P. aeruginosa* gDNA.

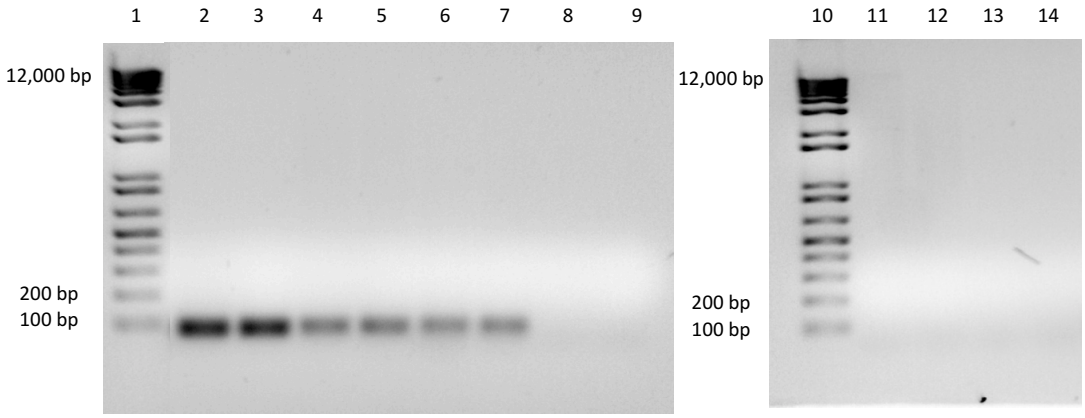


Figure 41. Validation of *E. coli* primers by agarose gel electrophoresis following qPCR with an annealing temperature of 60°C. Lanes 1, 10: 1 Kb Plus markers; Lanes 2, 3: *E. coli* gDNA; Lanes 4, 5: *S. mitis* gDNA; Lanes 6, 7: *S. pneumoniae* gDNA; Lanes 8,9: *S. aureus* gDNA; Lanes 11, 12: *K. pneumoniae* gDNA; Lanes 13, 14: *P. aeruginosa* gDNA.

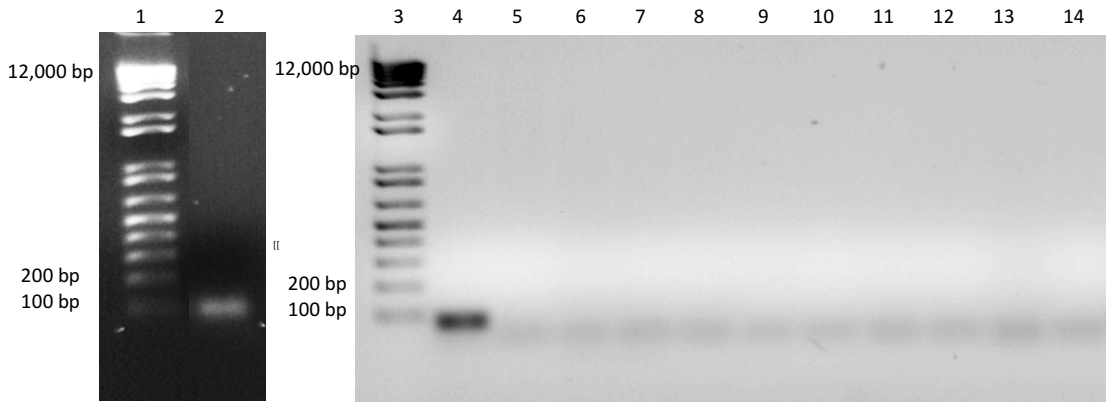


Figure 42. Validation of *K. pneumoniae* primers by agarose gel electrophoresis following qPCR with an annealing temperature of 60°C. Lanes 1, 3: 1 Kb Plus markers; Lanes 2, 4: *K. pneumoniae* gDNA; Lanes 5, 6: *S. mitis* gDNA; Lanes 7, 8: *S. pneumoniae* gDNA; Lanes 9, 10: *S. aureus* gDNA; Lanes 11, 12: *E. coli* gDNA; Lanes 13, 14: *P. aeruginosa* gDNA.

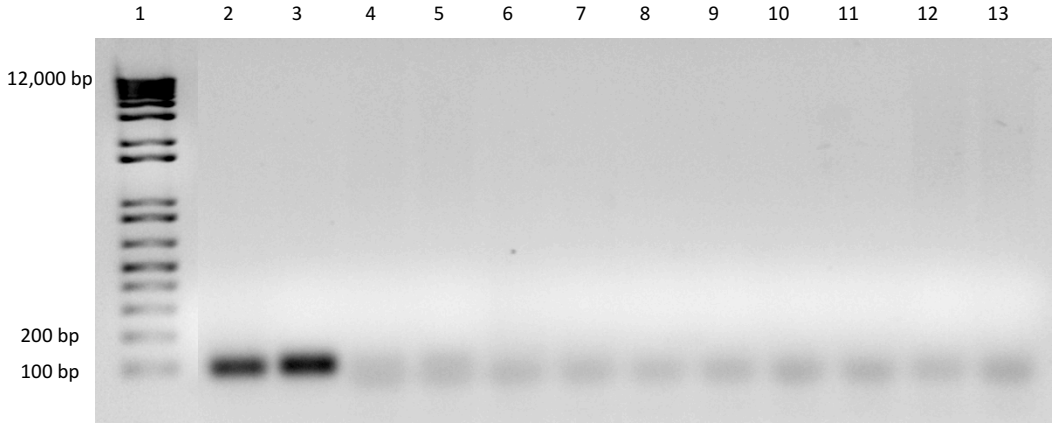


Figure 43. Validation of *P. aeruginosa* primers by agarose gel electrophoresis following qPCR with an annealing temperature of 60°C. Lane 1: 1 Kb Plus markers; Lanes 2, 3: *P. aeruginosa* gDNA; Lanes 4, 5: *S. mitis* gDNA; Lanes 6, 7: *S. pneumoniae* gDNA; Lanes 8, 9: *S. aureus* gDNA; Lanes 10, 11: *E. coli* gDNA; Lanes 12, 13: *K. pneumoniae* gDNA.

remaining primers, further end-point PCR using a temperature gradient protocol of 59 °C to 69 °C was undertaken. Visual inspection of the PCR products indicated that 63 °C was the optimal annealing temperature as this tended to be the final point before amplification was inhibited (Figures 44 - 47). The primer specificity experiment was repeated for *Streptococcus, S. pneumoniae, E. coli* and *P. aeruginosa* primers by qPCR. The qPCR cycling parameters were adjusted to 10 minutes at 95 °C and 40 cycles of 15 seconds at 95 °C and one minute at 63 °C. Inspection of the resulting amplification plots and melting curves indicated acceptable specificity of *Streptococcus, S. pneumoniae, E. coli* and *P. aeruginosa* primers at the new annealing temperature of 63 °C (Figures 48 - 55).

10.3.4. qPCR assay sensitivity.

Assay efficiencies are reported in Table 13. Schematic representations are also provided in Figures 57 and 57.

10.4. Results ii) Hypothesis Testing

Of the 102 participants included in the analysis: 102 completed the first outcome measurement, 95 completed the second outcome measurement and 94 completed the third outcome measurement. Clinical and demographic information is detailed in Table 14.

10.4.1. Changes in prevalence of target bacteria.

The relative levels of bacteria were not normally distributed so to categorise them, the median, upper and lower quartiles were calculated. For ease of interpretation, these are expressed in Table 15 as Cq values. Participants with relative levels of bacteria in the upper quartile were categorised as having ‘high’ amounts of bacteria, participants with relative bacteria levels in the lower quartile were characterised as having ‘low’

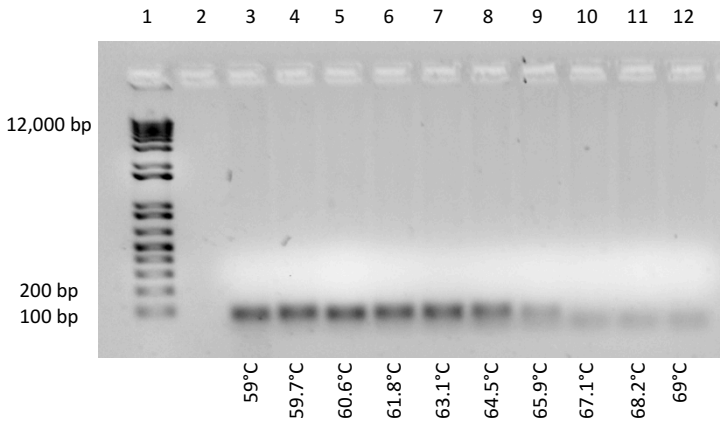


Figure 44. Investigation of optimal annealing temperature of a *Streptococcus* primer set using a temperature gradient protocol and amplicons visualised by agarose gel electrophoresis. Lane 1: 1 Kb Plus markers; Lanes 2 – 12: *Streptococcus* gDNA products. The various annealing temperatures are displayed below the gel image.

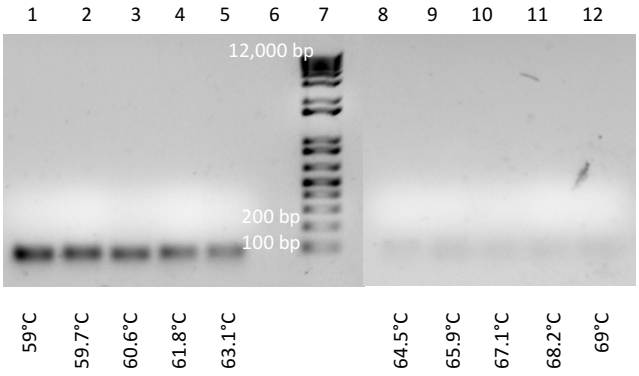


Figure 45. Investigation of optimal annealing temperature of *S. pneumoniae* primers using a temperature gradient protocol and amplicons visualised by agarose gel electrophoresis. Lane 7: 1 Kb Plus markers; Lanes 1 – 6, 8 – 12: *S. pneumoniae* gDNA products. The various annealing temperatures are displayed below the gel image.

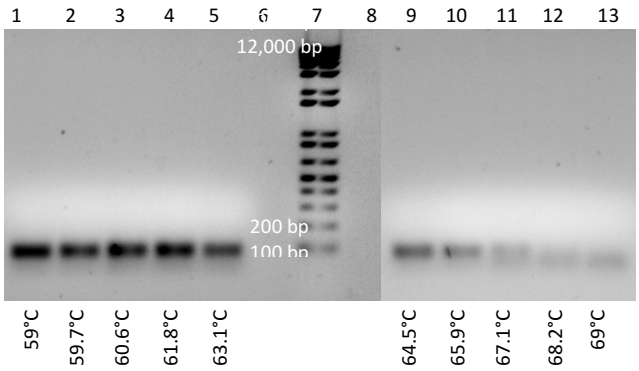


Figure 46. Investigation of optimal annealing temperature of *E. coli* primers using a temperature gradient protocol and amplicons visualised by agarose gel electrophoresis. Lane 7: 1 Kb Plus markers; Lanes 1 – 5, 9 – 13: *E. coli* gDNA products. The various annealing temperatures are displayed below the gel image.

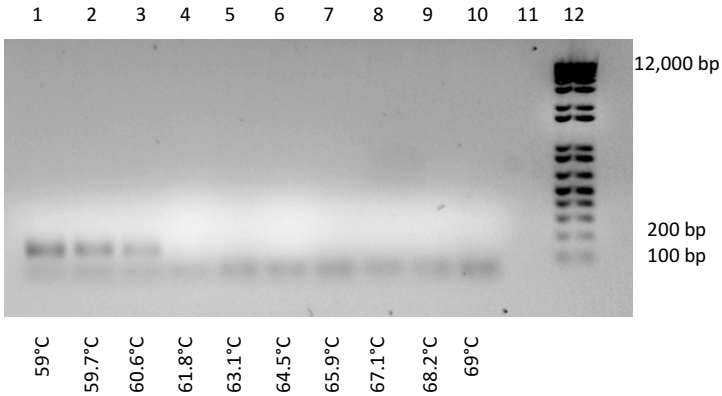


Figure 47. Investigation of optimal annealing temperature of *P. aeruginosa* primers using a temperature gradient protocol and amplicons visualised by agarose gel electrophoresis. Lane 12: 1kb Plus markers; Lanes 1 – 11: *P. aeruginosa* gDNA products. The various annealing temperatures are displayed below the gel image.

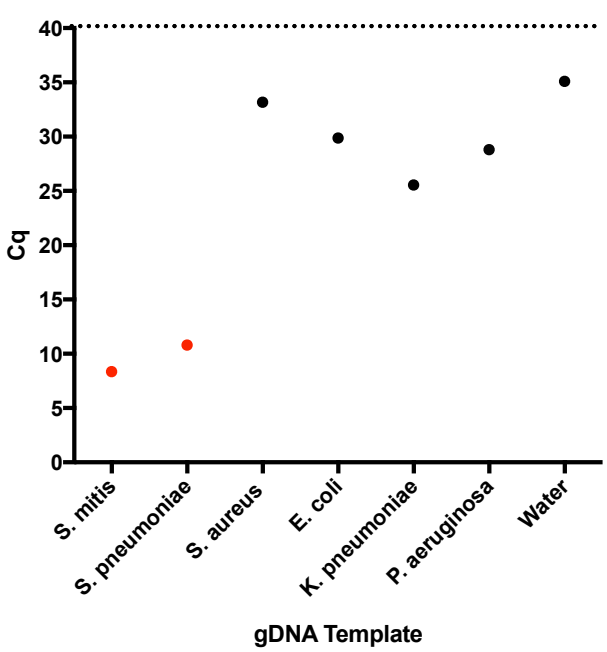


Figure 48. Amplification plot of a *Streptococcus* primer set against six bacterial standards at an annealing temperature of 63 °C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detectable limit of the assay.

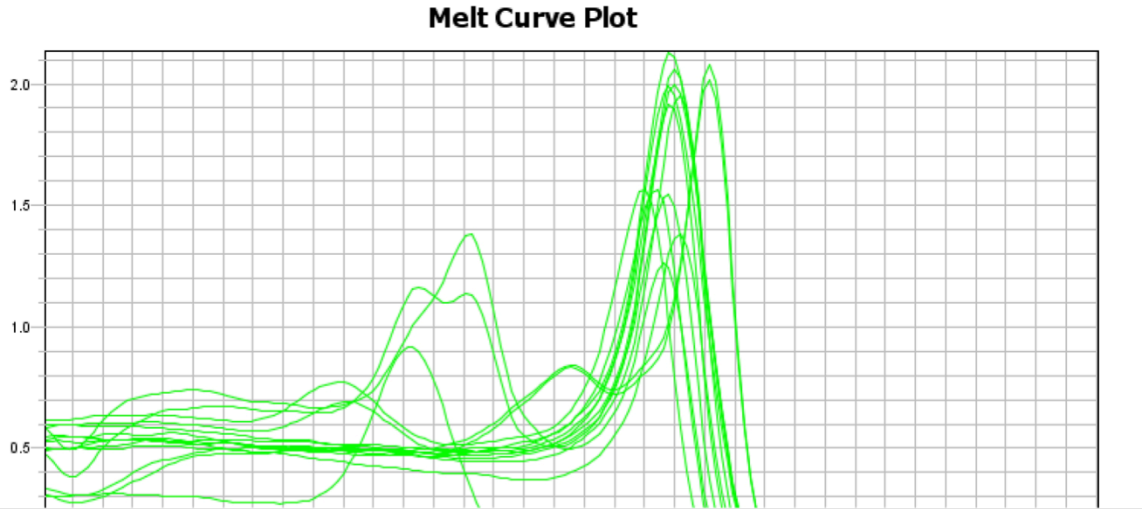


Figure 49. Melting curve plot depicting amplified gDNA products from six bacterial templates when a *Streptococcus* primer set were tested under annealing conditions of 63°C.

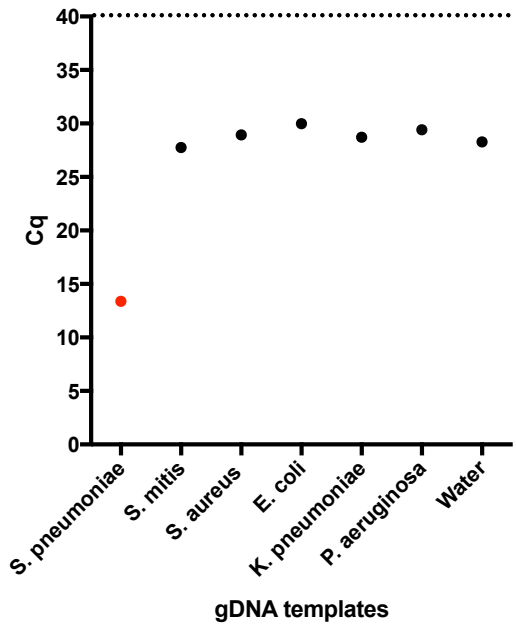


Figure 50. Amplification plot of *S. pneumoniae* primers against six bacterial standards at an annealing temperature of 63 °C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detectable limit of the assay.

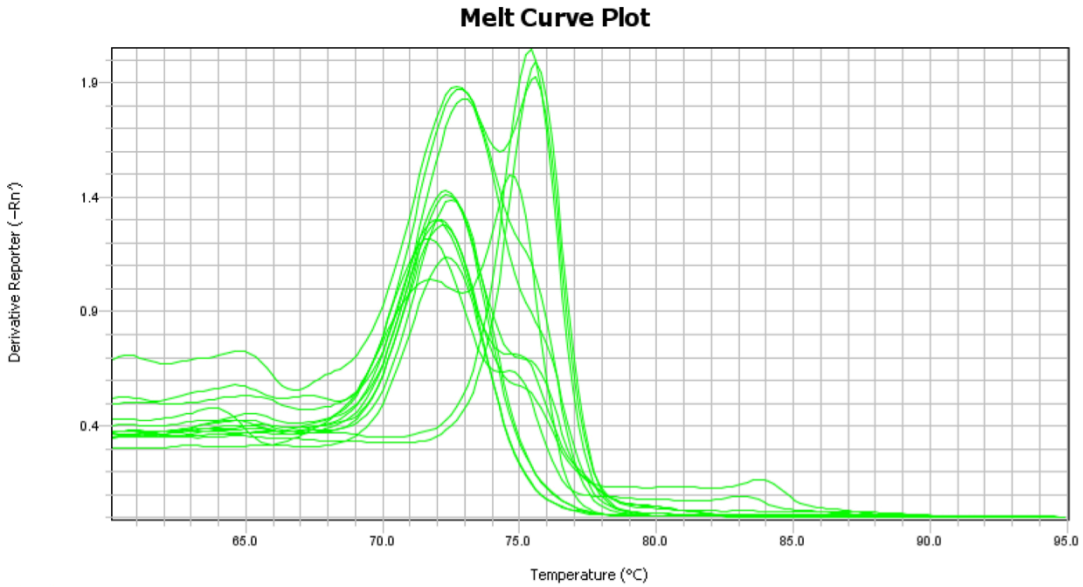


Figure 51. Melting curve plot depicting amplified gDNA products from six bacterial templates when *S. pneumoniae* primers were tested under annealing conditions of 63 °C.

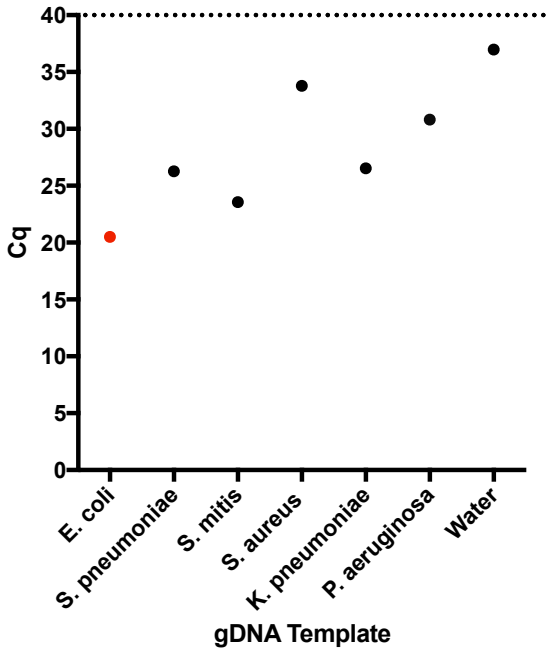


Figure 52. Amplification plot of *E. coli* primers against six bacterial standards at an annealing temperature of 63 °C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detectable limit of the assay.

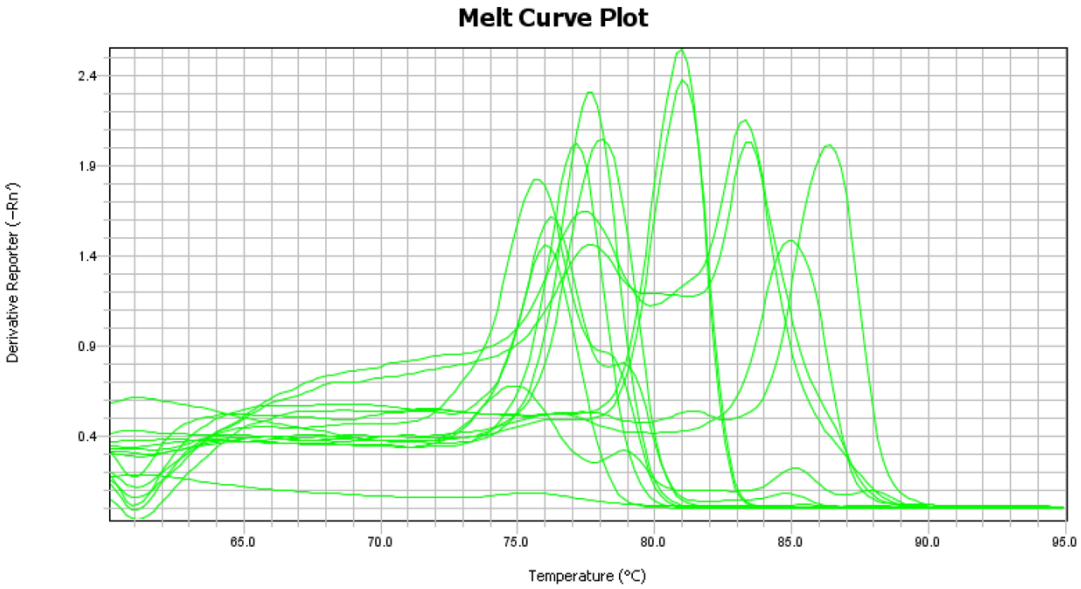


Figure 53. Melting curve plot depicting amplified gDNA products from six bacterial templates when *E. coli* primers were tested under annealing conditions of 63°C.

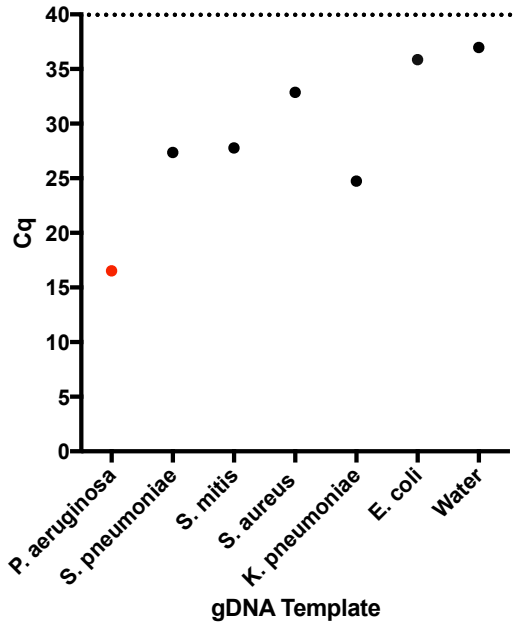


Figure 54. Amplification plot of *P. aeruginosa* primers against six bacterial standards at an annealing temperature of 63 °C. gDNA templates matching the primer set are highlighted in red. The dashed line represents the detectable limit of the assay.

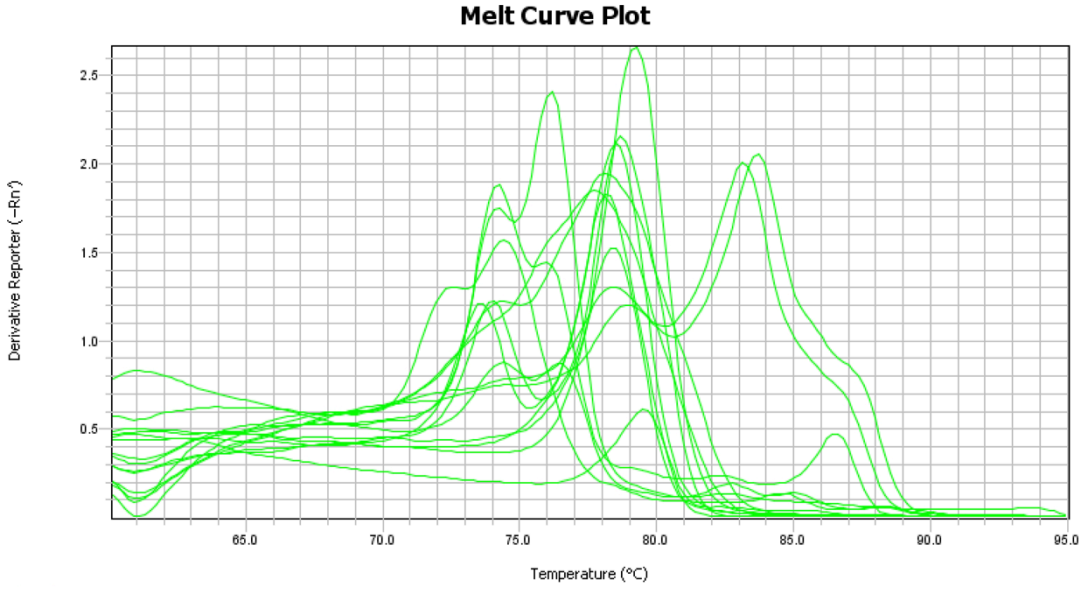


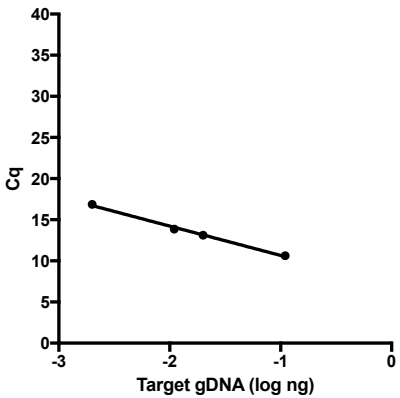
Figure 55. Melting curve plot depicting amplified gDNA products from six bacterial templates when *P. aeruginosa* primers were tested under annealing conditions of 63 °C.

Table 13

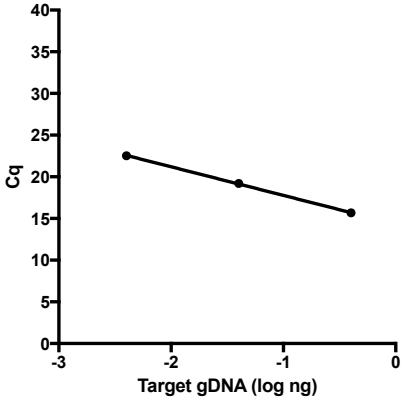
Assay Efficiencies for Detecting Streptococcus, S. pneumoniae, S. aureus, E. coli, K. pneumoniae and P. aeruginosa

Assay/target bacteria	qPCR efficiency (%)	Slope	R^2
Streptococcus	90.60	-3.57	1.00
S. pneumoniae	95.64	-3.43	0.72
S. aureus	91.32	-3.55	1.00
E. coli	92.60	-3.51	0.80
K. pneumoniae	93.62	-3.49	1.00
P. aeruginosa	92.35	-3.52	1.00

A



B



C

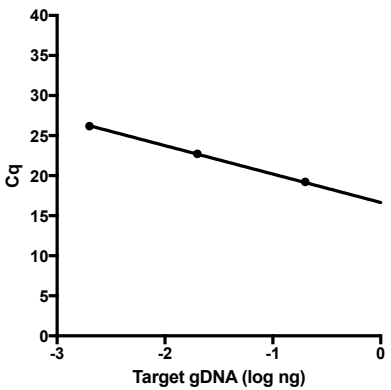
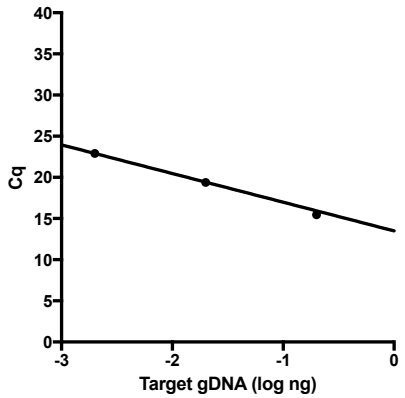
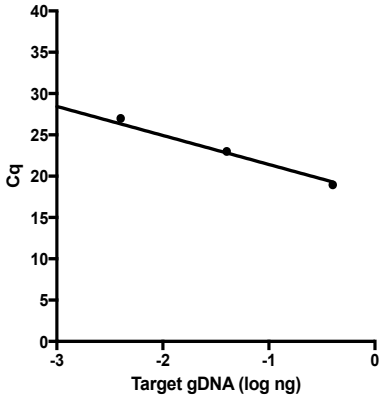


Figure 56. qPCR assay sensitivity for *Streptococcus* (A), *S. pneumoniae* (B) and *S. aureus* (C). Assay (A) used *Streptococcus* primers and achieved a 90.60 % amplification efficiency (slope = -3.57, R^2 = 1.00) with a five-fold dilution series of 0.002 – 0.11 ng/well of gDNA template. Assay (B) used *S. pneumoniae* primers and achieved a 95.64 % amplification efficiency (slope = -3.43, R^2 = 0.72) with a five-fold dilution series of 0.4 – 0.04 ng/well of gDNA template. Assay (C) used *S. aureus* primers and achieved a 91.32 % amplification efficiency (slope = -3.55, R^2 = 1.00) with a ten-fold dilution series of 0.002 – 2 ng/well of gDNA. The mean Cq and standard error of the mean are displayed. No error bars are visible due to the small differences between duplicate wells.

D



E



F

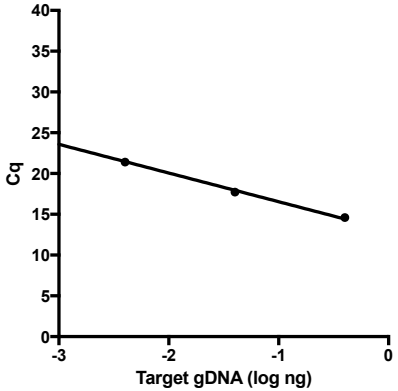


Figure 57. qPCR assay sensitivity for *K. pneumoniae* (D), *E. coli* (E) and *P. aeruginosa* (F). Assay (D) used *K. pneumoniae* primers and achieved a 93.62 % amplification efficiency (slope = -3.49, R^2 = 1.00) with a ten-fold dilution series of 0.002 – 2 ng/well of gDNA template. Assay (E) used *E. coli* primers and achieved a 92.60 % amplification efficiency (slope = -3.51, R^2 = 0.80), with a ten-fold dilution series of 0.0004 – 0.4 ng/well of gDNA template. Assay (F) used *P. aeruginosa* primers and achieved a 92.35 % amplification efficiency (slope = -3.52, R^2 = 1.00) with a ten-fold dilution series of 0.0004 – 0.4 ng/well of gDNA template. The mean Cq and standard error of the mean are expressed. No error bars are visible due to the small difference between duplicate wells.

Table 14

Clinical and Demographic Information of Participants

Clinical/Demographic Variable	*n*
Age (years)	Mean = 76, *SD* = 12.5
Gender	
Male	61 (60 %)
Female	41 (40 %)
Ethnicity	
New Zealand European	85 (83 %)
European	11 (11 %)
Chinese	2 (2 %)
New Zealand Maori	2 (2 %)
Indian	1 (1 %)
Niuean	1 (1 %)
Comorbidities	
Previous stroke	27 (26 %)
Respiratory illness	21 (21 %)
Cardiac illness	58 (57 %)
Dementia	4 (4 %)
Diabetes Mellitus	19 (19 %)
Type of stroke	
Ischaemic	89 (87 %)
Haemorrhagic	11 (11 %)
Ischaemic with haemorrhagic transformation	1 (1 %)
Not reported	1 (1 %)

Site of lesion	
Supratentorial	88 (86 %)
Infratentorial	11 (11 %)
Mixed	2 (2 %)
Not reported	1 (1 %)
Lesion laterality	
Left	42 (41 %)
Right	56 (55 %)
Bilateral	3 (3 %)
Not reported	1 (1 %)
Independent for oral hygiene at admission*	55 (54 %)
Independent for oral hygiene at discharge*	74 (78 %)
Independent for oral hygiene at one month*	78 (83 %)
Days between admission to hospital and first data collection (median and range)	2, 33
Days between discharge from hospital and second data collection (median and range)	0, 11**
Days between stroke and third data collection (median and range)	0, 11
Days on acute hospital ward† (median and range)	5, 12
More than 30 days in hospital*†	30
Suspected/diagnosed AP on admission*	6 (6 %)
Developed AP during hospital admission*	11 (11 %)
Incidence of AP over one month period	11 (11 %)
Mortality within one month of stroke	9 (9 %)

Cause of death:	
Pneumonia	5 (5 %)
Other (stroke, cardiac disease)	4 (4 %)

Note. †Excluding participants who died while in hospital, *SD* = standard deviation, * refers to the number of participants, ** refers to the number of days prior to discharge from hospital, AP = aspiration pneumonia

Table 15

Descriptive Analysis of Levels of Target Bacteria Expressed as Cq Values at Three Measurement Points

Target bacterial species	Measurement Point	Lower quartile	Median Cq	Upper quartile
Streptococcus	Admission	17.74	18.63	21.39
	Discharge	17.92	20.85	26.13
	One Month	18.18	19.99	21.93
S. pneumoniae	Admission	24.79	25.74	26.30
	Discharge	23.43	25.63	26.95
	One Month	24.92	26.23	26.62
S. aureus	Admission	40	40	40
	Discharge	26.85	28.09	29.34
	One Month	27.85	27.85	27.85
E. coli	Admission	17.17	17.27	17.37
	Discharge	40	40	40
	One Month	40	40	40
K. pneumoniae	Admission	40	40	40
	Discharge	29.36	29.53	29.71
	One Month	40	40	40
P. aeruginosa	Admission	29.42	29.85	30.18
	Discharge	27.23	29.68	30.33
	One Month	29.71	30.01	30.39

amounts of bacteria and participants with relative bacteria levels within this range were characterised as having 'medium' amounts of bacteria.

Eighteen participants (18 %) had no detectable pathogens at any sampling point, 25 participants (25 %) had at least one pathogen at all three sample times and one participant (P46; 1 %) had two pathogens present at all three time points. The proportion of participants with detectable levels of pathogenic bacteria at each sampling location is depicted in Figure 58. There was a significant association between the proportion of patients with detectable levels of pathogenic bacteria and the patients' location at the time of sampling [χ^2 (2) = 6.38, p = .04]. Specifically, the greatest proportion of detectable oral pathogens was from patients sampled on an acute hospital ward.

Streptococcus were the most commonly detected bacteria, whereas *S. aureus, K. pneumoniae* and *E. coli* were extremely rare (Figure 59). Levels of bacteria were not significantly different at the different time points [F(1.11, 7.74) = 0.45, p = .54, 1 – β = .09] (Figure 60). Epsilon (ε) was 0.55, as calculated according to Greenhouse & Geisser (1959) and was used to correct the one-way repeated measures ANOVA. Because fourteen participants discharged within 24 hours of baseline testing, the same data were used for their discharge measures. A second one-way repeated-measures ANOVA was run to determine if excluding these participants from the dataset made a difference to the ability to measure changes in bacteria levels over time. The second model was not statistically significant [F(1.03, 5.17) = 0.26, p = .64, 1 – β = .11].

None of the six target bacteria displayed a significant change in prevalence at the three measurement points (Table 16). Repeated testing excluding those participants who contributed the same data at admission and discharge did not alter this.

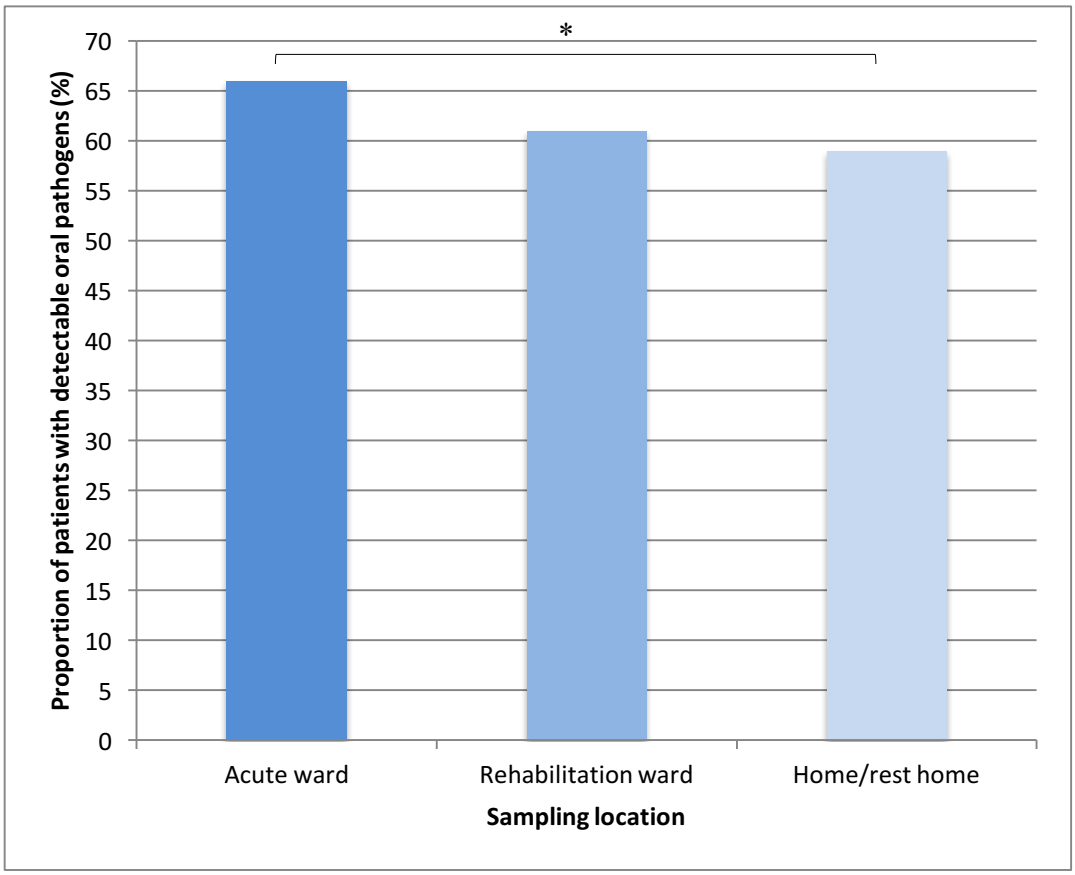


Figure 58. The proportion of participants with detectable levels of pathogenic bacteria at three sampling locations. * $p = .03$

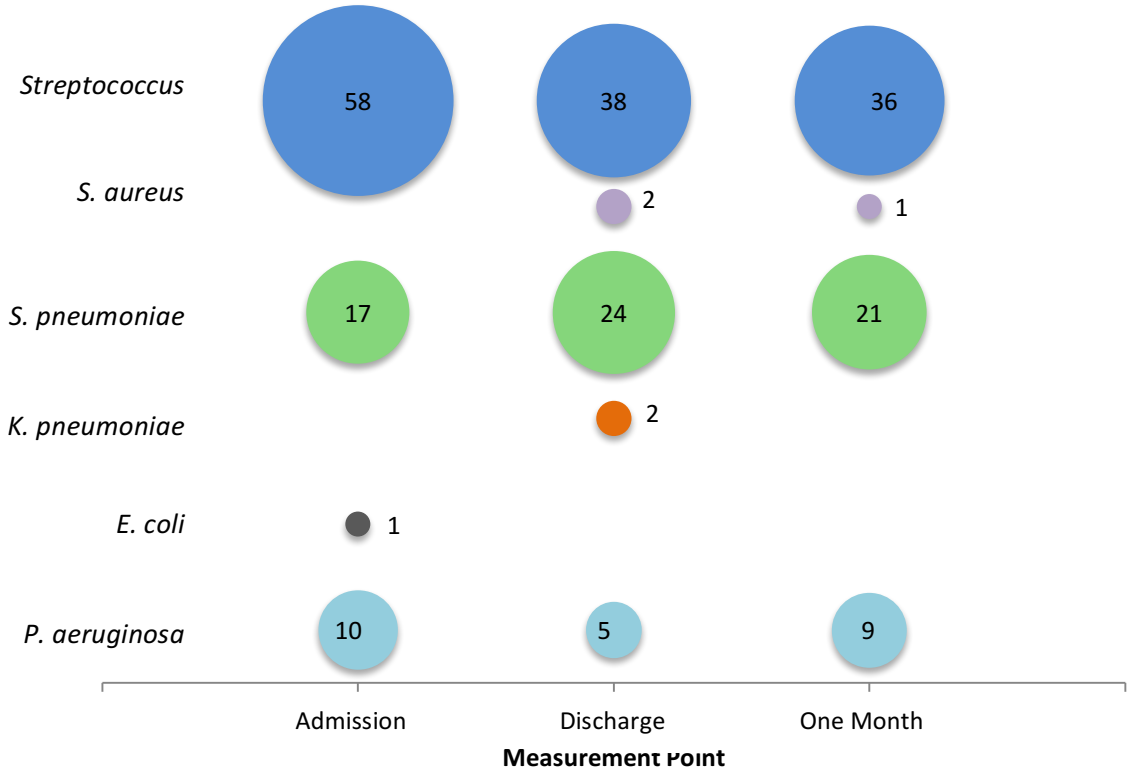


Figure 59. Prevalence (%) of target organisms in saliva samples at three sample times

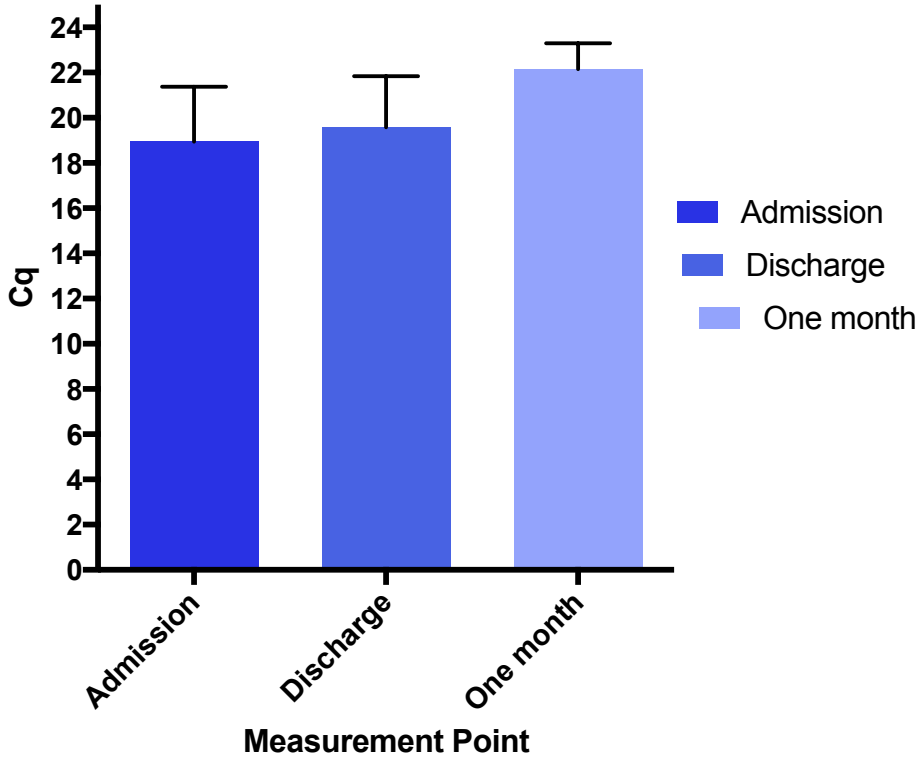


Figure 60. Cq values of all target bacteria at three measurement points (median and 95 % *CI*). Cq values are not significantly different across measurement points.

Table 16

Prevalence of Six Target Bacteria at Three Measurement Points

Bacteria	Mean Cq at baseline	Mean Cq at discharge	Mean Cq at one month	b	*SE* b	*p*	95 % *CI*
Streptococcus	20.63	22.32	20.32	20.52	0.91	.06	19.71 – 22.34
S. pneumoniae	25.43	25.16	25.56	-0.27	0.37	.46	-1.00 – 0.45
S. aureus	40	28.09	27.85	-0.06	0.07	0.44	-0.23 – 0.11
E. coli	17.27	N.D	N.D	0.11	0.09	0.22	-0.07 – 0.29
K. pneumoniae	N.D	29.53	N.D	N/A	N/A	N/A	N/A
P. aeruginosa	29.4	28.92	29.98	0.08	0.21	0.71	-0.34 – 0.49

Note. CI = confidence interval, N.D = not detected. The occurrence of *K. pneumoniae* was too low for linear mixed modelling.

The logistic regression model did not significantly predict levels of total bacteria at admission to hospital [$p = .09$] or discharge from hospital [$p = .27$]. At one month, the model did not significantly predict total bacteria levels [$p = .08$].

Further logistic regression modelling did not significantly predict levels of *Streptococcus* at admission [$p = .75$], discharge [$p = .44$] or one month [$p = .37$]. There was a significant main effect of gender on levels of *S. pneumoniae* at admission [$\chi^2(2) = 8.47$, $p = .01$], with being female associated with higher levels of this bacterium. The model did not predict levels of *S. pneumoniae* at discharge [$p = .17$] or one month [$p = .94$]. The model did not predict levels of *S. aureus* at discharge [$p = .32$] or one month [$p = .17$] and levels of this bacterium at admission were too low for analysis. Levels of *K. pneumoniae* were also too low for analysis. The model did not predict levels of *P. aeruginosa* at admission [$p = .32$] or one month [$p = .20$]. Gender had a significant main effect on levels of *P. aeruginosa* at discharge [$\chi^2(2) = 9.26$, $p = .01$], with females more likely to have medium or high levels of bacteria compared to males. There was also a main effect of age, with younger patients more likely to have medium or high levels of this bacterium at discharge [$\chi^2(2) = 9.39$, $p = .01$]. The model did not predict levels of *E. coli* at admission [$p = .43$], with levels of this bacterium at discharge and one month too low for analysis.

10.4.2. Changes in cough reflex sensitivity over time.

Changes in cough reflex thresholds are displayed in Figure 61. Data were not normally distributed. Cough reflex thresholds remained identical across admission, discharge and at one month [Median = 0.40 mol/L]. Cough reflex thresholds did not significantly differ by time point [$F(2, 166) = 0.12$, $p = .88$, $1 - \beta = .08$]. When participants who discharged within 24 hours of baseline testing were excluded, there remained no significant differences in cough reflex thresholds over time [$F(2, 138) =$

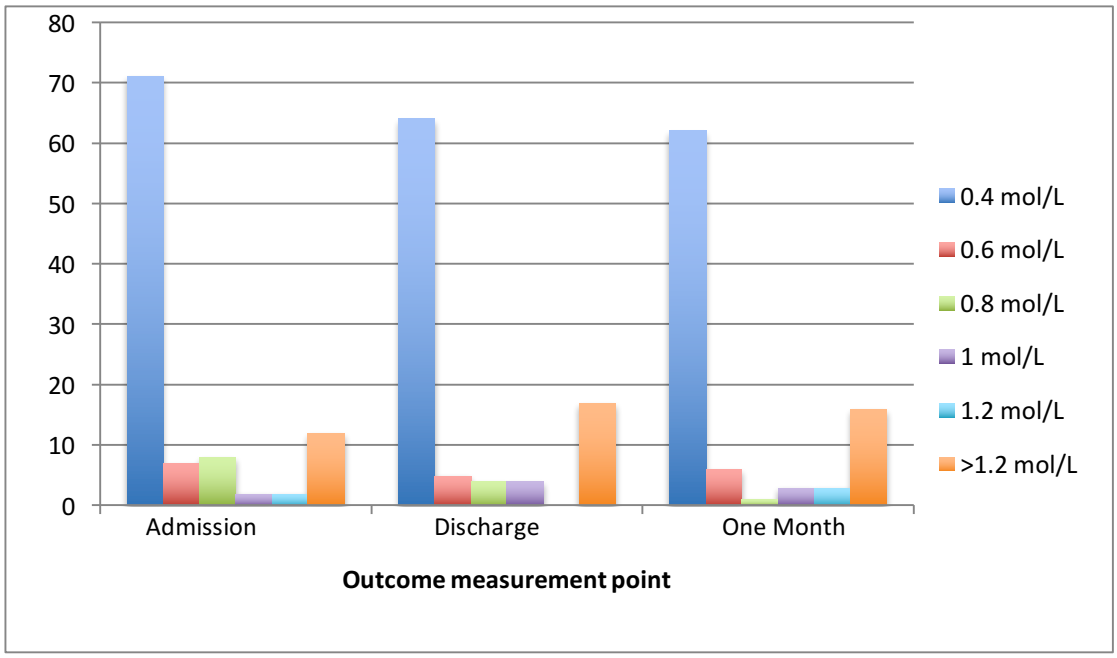


Figure 61. Distribution of Cough Reflex Thresholds Over Three Outcome Measurement Points

0.05, $p = .95$].

The multiple regression model significantly predicted baseline cough reflex thresholds [F(12, 100) = 2.52, $p = .007$, $1 - \beta = .78$]. R^2 for the first model was 11 %, indicating a small effect size (Cohen, 1988). The addition of bacteria levels and time of testing as predictors of cough reflex thresholds increased the R^2 to 26 %, suggesting that these variables accounted for an additional 15 % of the variance in cough reflex thresholds. Gender was revealed as a significant predictor of baseline cough reflex thresholds, with being female associated with higher cough reflex thresholds [$p = .02$]. Regression coefficients and standard errors can be found in Table 17.

At discharge, the basic linear regression model significantly predicted cough reflex thresholds [$F(3, 92) = 3.32$, $p = .02$, $1 - \beta = .11$], however no individual predictor emerged as significantly associated with cough reflex thresholds. The addition of time of testing and bacteria levels as predictors did not significantly improve the model [$F(13, 92) = 1.39$, $p = .18$, $1 - \beta = .72$]. The overall model had an R^2 of 19 %, indicating a small effect (Cohen, 1988). Regression coefficients and standard errors can be found in Table 18.

At one month, the basic linear regression model significantly predicted cough reflex thresholds [$F(3, 89) = 3.49$, $p = .02$, $1 - \beta = .08$], however no individual predictor emerged as significantly associated with cough reflex thresholds. The addition of time of testing and bacteria levels as predictors did not significantly improve the model [$F(12, 89) = 1.45$, $p = .16$, $1 - \beta = .14$]. The overall model had an R^2 of 11 %, indicating a small effect (Cohen, 1988). Regression coefficients and standard errors can be found in Table 19. The relationship between cough reflex sensitivity and levels of each bacteria is depicted in Figure 62.

Table 17

Multiple Regression Models Predicting Cough Reflex Thresholds on Admission to Hospital

	B	*SE B*	β	*p*
Step 1				
Constant	.84	.22		.00
Age	-.01	.00	-.18	.09
Gender	.14	.06	.22	**.03***
Smoking	.07	.10	.07	.51
Step 2				
Constant	.61	.23		.01
Age	-.00	.00	-.12	.25
Gender	.16	.06	.24	**.02***
Smoking	.05	.11	.05	.66
High levels of *Streptococcus*	.03	.07	.04	.66
Moderate levels of *Streptococcus*	.16	.09	.18	.07
High levels of *S. pneumoniae*	.15	.12	.14	.20
Moderate levels of *S. pneumoniae*	.05	.14	.03	.74
High levels of *E. coli*	.51	.32	.16	.12
High levels of *P. aeruginosa*	.24	.14	.18	.09
Moderate levels of *P. aeruginosa*	-.07	.22	-.03	.74
Testing was at noon	.13	.07	.18	.08

Testing was in the afternoon	.07	.09	.08	.44

Note. R^2 = .11 for Step 1 ($p < .05$), ΔR^2 = .15 for Step 2 ($p < .01$). * $p < .05$

Table 18.

Results from Multiple Regression Models Predicting Cough Reflex Thresholds at Discharge from Hospital

	B	*SE B*	β	*p*
Step 1				
Constant	.81	.25		.00
Age	-.00	.00	-.14	.21
Gender	.13	.07	.18	.08
Smoking	.17	.12	.15	.16
Step 2				
Constant	.59	.29		.04
Age	-.00	.00	-.05	.67
Gender	.15	.08	.21	.07
Smoking	.21	.13	.19	.11
High levels of *Streptococcus*	.01	1.00	.01	.92
Moderate levels of *Streptococcus*	-.22	.15	-.16	.14
High levels of *S. pneumoniae*	.14	.27	.06	.61
Moderate levels of *S. pneumoniae*	.09	.12	.09	.43
High levels of *P. aeruginosa*	.16	.22	.08	.48
Moderate levels of *P. aeruginosa*	.28	.38	.08	.47
High levels of *S. aureus*	-.22	.35	-.06	.54
Moderate levels of *S. aureus*	-.22	.35	-.06	.53
Testing was at noon	-.09	.10	-.11	.35

Testing was in the afternoon	.18	.10	.19	.10

Note. $R^2 = .10$ for Step 1 ($p < .05$), $\Delta R^2 = .09$ for Step 2 ($p > .05$).

Table 19.

Results from Multiple Regression Models Predicting Cough Reflex Thresholds at One Month Post-stroke

	B	*SE B*	β	*p*
Step 1				
Constant	.90	.26		.00
Age	-.01	.00	-.16	.14
Gender	.10	.08	.12	.21
Smoking	.21	.12	.19	.09
Step 2				
Constant	.82	.27		.00
Age	-.00	.00	-.11	.35
Gender	.12	.08	.15	.15
Smoking	.22	.13	.20	.09
High levels of *Streptococcus*	-.15	.11	-.15	.20
Moderate levels of *Streptococcus*	.05	.15	.04	.75
High levels of *S. pneumoniae*	-.02	.13	-.02	.90
Moderate levels of *S. pneumoniae*	.18	.17	.11	.30
High levels of *P. aeruginosa*	.29	.20	.17	.14
Moderate levels of *P. aeruginosa*	.21	.22	.11	.34
High levels of *S. aureus*	-.15	.37	-.04	.69
Testing at noon vs. in the morning	-.11	.10	-.13	.28
Testing was in the afternoon vs.	-.09	.09	-.12	.31

morning

Note. R^2 = .11 for Step 1 ($p < .05$), ΔR^2 = .08 for Step 2 ($p > .05$).

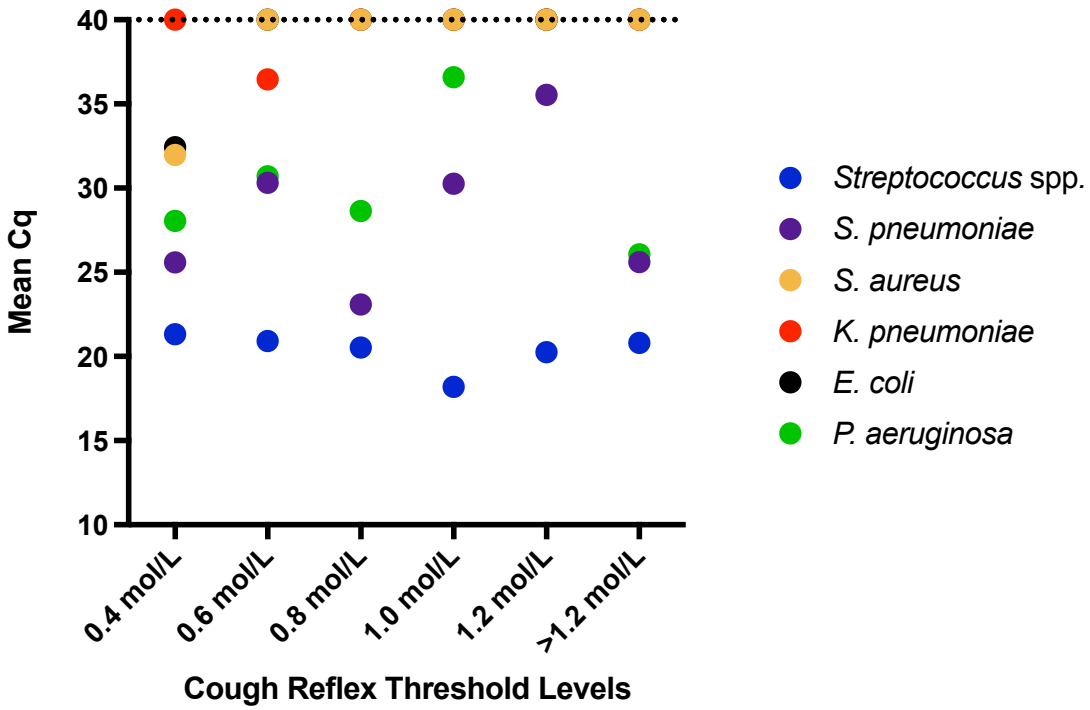


Figure 62. The relationship between mean bacteria levels (expressed as Cq) and cough reflex sensitivity levels. The dashed line represents the assay limit of detection and points on this line indicate that no bacteria were detected.

10.4.3. Prevalence of aspiration pneumonia.

Six participants (6 %) presented to hospital with suspected pneumonia, eleven participants (11 %) developed AP during hospitilisation with one of these participants also testing positive for influenza. There were no new diagnoses of AP following discharge.

The multinomial logistic regression models did not predict the occurrence of AP at admission to hospital regardless of whether target bacteria were combined [$\chi^2(5) = 2.63$, $p = .76$] or categorised individually [$\chi^2(10) = 7.42$, $p = .69$]. Similarly, the regression models did not predict the occurrence of AP at discharge from hospital regardless of whether target bacteria were combined [$\chi^2(5) = 4.74$, $p = .45$] or categorised individually [$\chi^2(11) = 13.66$, $p = .25$]. The relationship between overall levels of bacteria over time and the incidence of aspiration pneumonia in all participants is displayed in Figure 63. The relationship between specific bacteria levels over time in participants who developed AP is displayed in Figure 64.

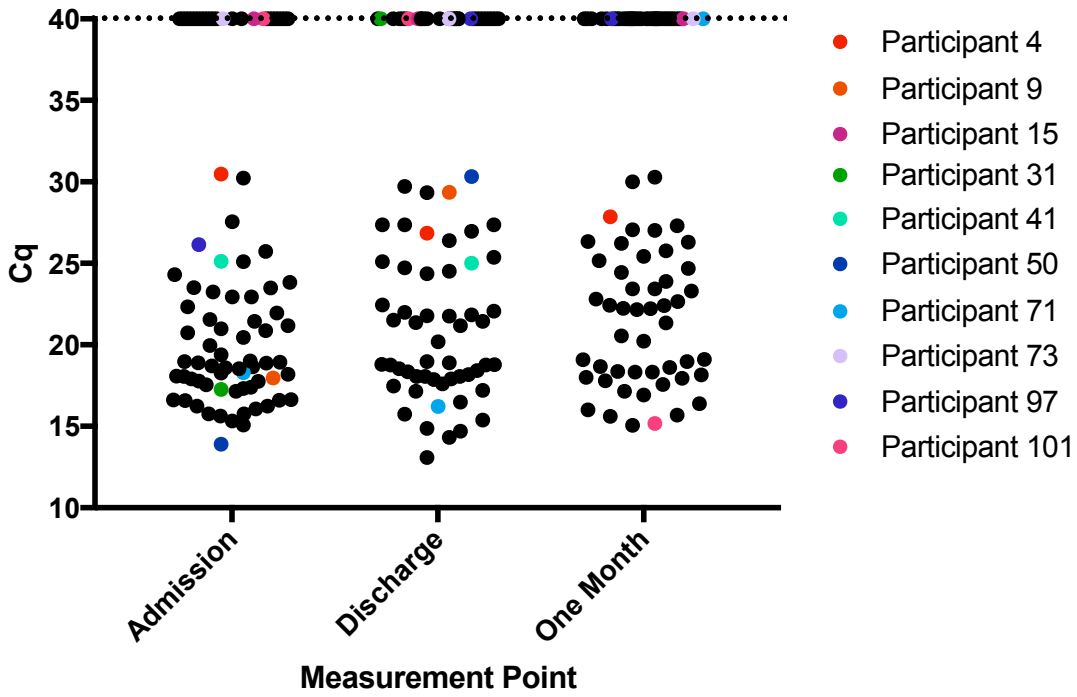


Figure 63. The relationship between bacteria numbers (expressed as Cq) across all target bacteria and time in participants with acute stroke and dysphagia. Participants who developed aspiration pneumonia are indicated in colour. The dashed line represents the detection limit of the assay. Points on the dashed line indicate that no bacteria were detected.

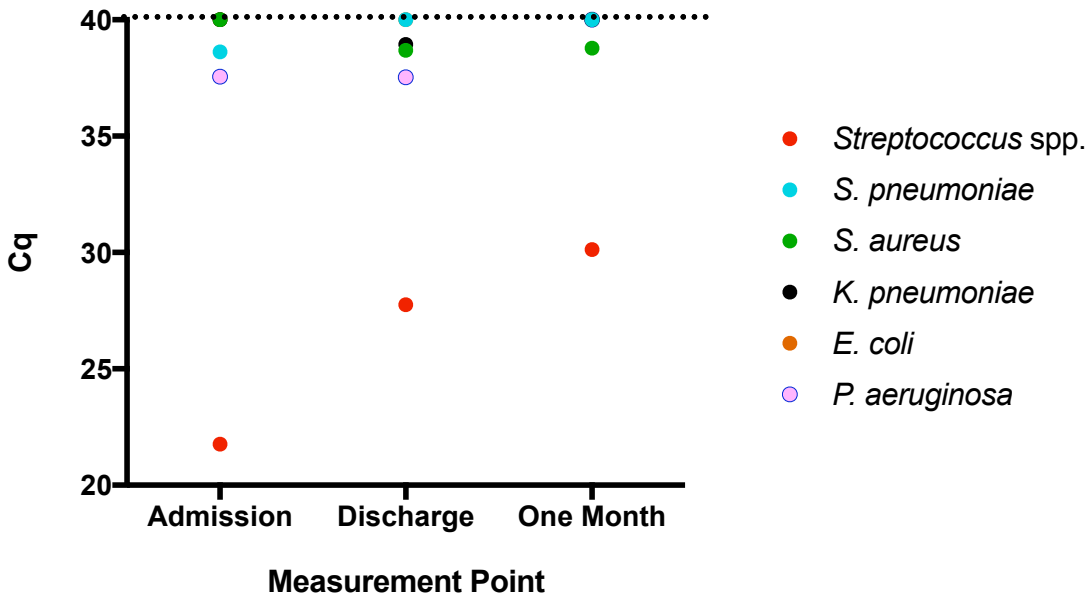


Figure 64. The relationship between levels of target bacteria (expressed as Cq) at different sampling points among patients who developed AP.

Chapter 11. Discussion

The aim of this thesis was to investigate contributing factors to the development of AP among patients with acute post-stroke dysphagia and evaluate methods in which AP may be prevented in this population. For the first time, diurnal variation in reflexive cough sensitivity was investigated using the facemask/tidal breathing method. No evidence of a diurnal effect was found, challenging current views in CRT methodology. Adaptation to the test, as described in similar studies, was confirmed. This has implications for clinicians and researchers who use repeated outcome measurements.

This is the first record of the effects of the Dysphagia in Stroke Protocol (DiSP) on functional patient outcomes. The DiSP is unique in that it incorporates standardised management guidelines, screening for silent aspiration, instrumental assessment and CRT methods that are appropriate for a neurologically-impaired population. A significant reduction in AP was observed, as well as improvements in LOS, post-stroke independence and diet level tolerance. Despite significantly improved patient outcomes, other variables not accounted for in this study appear to influence these positive outcomes. The extent to which these results can be attributed to the DiSP is unclear and warrants further investigation.

A relationship between oral bacteria, cough reflex sensitivity and AP in this population could not be confirmed. A low observed rate of AP and difficulties recruiting patients with severe stroke-related impairment contributed to low statistical power. The hypothesis that oral bacteria levels are related to cough reflex sensitivity and AP is theoretically sound and studies with larger sample sizes may reveal this.

Data from the present study represent a valuable contribution to future study designs and power calculations.

11.1. Cough Reflex Testing Methodology

Although CRT has been used in the field of respiratory medicine for over 60 years (Bickerman et al., 1954; Bickerman, German, Cohen, & Itkin, 1957), its application to the field of speech-language therapy is relatively recent. Given the wide variability in CRT methods, including tussive agents, nebulisers, method of administration and outcome measurement, it is vital that users of the CRT understand its limitations, including test reproducibility and confounding variables.

Since Pounsford and Saunders (1985) first reported diurnal variation in cough reflex sensitivity, time of testing has been considered a major confounding variable (Morice et al., 2001; Nakajoh et al., 2000) in clinical research. However, diurnal variation has only been described in CRT using a vital-capacity method. Study III aimed to measure variation in cough reflex sensitivity at different times of the day in healthy young adults using the tidal-breathing method that is commonly used in clinical assessment and attempting to control for oral bacteria. It was hypothesized that there would be no significant differences in cough reflex sensitivity when measured at different times of the day.

11.2. Diurnal Variation in Cough Reflex Sensitivity.

No evidence of diurnal variability in cough reflex sensitivity was observed and the null hypothesis was rejected. This is in direct contrast to the findings reported by Pounsford and Saunders (1985). One possible explanation for this difference could be related to oral bacteria levels. While the present study controlled for oral bacteria at a basic level by having participants brush their teeth prior to each testing session, the historical study by Pounsford and Saunders did not. Watando et al. (2004)

demonstrated significantly improved reflexive cough thresholds among elderly rest home residents who underwent an intensive oral hygiene regime, although the mechanism for this improvement remains unknown. In Study III, an attempt was made to characterise the relationship between pathogenic oral bacteria and cough reflex sensitivity, however findings were limited by low statistical power and no firm conclusions were drawn. To date, there remains a lack of empirical research that adequately describes the relationship between oral bacteria and cough reflex sensitivity. However, given that Pounsford and Saunders found significantly higher cough thresholds over the course of the day as oral bacteria levels presumably increased, it is possible that the true variable of influence in their study was oral bacteria and not time of day.

The average SCT in the present study was 0.6 mol/L (morning and afternoon tests) compared to ~ 0.2 mol/L (morning) and ~ 0.4 mol/L (afternoon) reported by Pounsford and Saunders (1985). Normative data established using the tidal breathing/face mask method suggest that the majority of young, healthy adults have a suppressed cough reflex threshold of 0.8 mol/L (Monroe et al., 2014). The reason for these discrepancies is most likely due to methodological differences. It is well-established that differences in CRT administration can affect the deposition of drug particles throughout the respiratory tract. This can be a function of nebuliser flow rate (Barros et al., 1990), administration of the aerosol (Wolfsdorf et al., 1969), individual respiratory rate, irritation and coughing (Barros et al., 1990; Pounsford & Saunders). For example, presenting the aerosol via a mouthpiece (e.g. Pounsford & Saunders) is likely to deposit the majority of particles in the lower respiratory tract (Wolfsdorf et al.), compared to presentation via a facemask (e.g. the present study and Monroe et al.), which deposits the majority of particles in the upper respiratory tract (Wolfsdorf

et al.). Such differences in methodology between Pounsford and Saunders and the present study are the obvious source of the differing cough reflex thresholds, but do not explain why Pounsford and Saunders observed diurnal variation using their CRT method. Further investigation is required to satisfactorily answer this question.

Eleven participants (21 %) failed to trigger a SCT during either assessment. This number is consistent with normative data (Monroe et al., 2014). It is unlikely that the present study suffered from a 'ceiling effect', as citric acid concentrations of up to 1.2 mol/L were presented to participants. According to norms, 96 % of healthy individuals produce a SCT by this concentration (Monroe et al.).

11.3. Reproducibility of the Cough Reflex Test.

A significant order effect was identified in SCTs that was not related to time of testing, supporting the theory that a degree of supramedullary influence over the cough reflex is possible in healthy participants (Hegland et al., 2012). This is in line with previous reports of a learning effect in CRT (Bickerman et al., 1954; Bickerman, Cohen, German, & Itkin, 1956; Pounsford & Saunders, 1985), with second presentations resulting in higher cough reflex thresholds (Hoffmeyer et al., 2013; Morice et al., 1992) as subjects learn to exert greater control over their response.

It has long since been recognised that in order for CRT to be clinically useful, subjects must respond in a uniform and consistent manner and there must be within-subject reproducibility (Bickerman et al., 1954). Cough response to citric acid is reproducible within one to three dose levels when measured on the same day (Barber et al., 2005; Bickerman et al., 1957; Wright, Jackson, Thompson, & Morice, 2010) and within one dose level when measured within the same week (Pounsford & Saunders, 1985) and the same month (Di Franco et al., 2001; Rees & Clark, 1983; Schmidt, Jörres, & Magnussen, 1997; Wright et al., 2010), with the exception of one

study using a Mefar dosimeter protocol (Wright et al.). However, the definition of "dose level" differs widely between studies. This can be problematic for clinical application, where a difference of one dose level may constitute the difference between a pass/fail on clinical examination (Addington, Stephens, & Gilliland, 1999; Miles et al., 2013a). Standardisation of CRT methods would greatly improve the ability to synthesize results across studies and apply the findings to clinical populations. Despite the limitations of current CRT methods, the test has high reported sensitivity and specificity for detecting silent aspiration in clinical populations (Miles et al., 2013b; Wakasugi et al., 2008, 2014) and, as discussed in Study I, when CRT is incorporated into a standardised management protocol there is an associated reduction in AP. Issues with test-retest reliability may be less relevant for clinical populations who typically only require a single test.

The only identified data regarding the reproducibility of citric acid CRT using a facemask method is from 1957 (Bickerman et al.) where reproducibility was measured over only four hours. In 2007, the European Respiratory Society (Morice et al., 2007) called for standardisation of all CRT methods and increased information regarding the reproducibility of CRT. This request remains to be satisfied. In the meantime, clinicians wanting to incorporate CRT into their clinical practice must pay close attention to CRT methodology and be cautious in their interpretation of results from repeated CRTs. Based on the findings of the present study and others (Hoffmeyer et al., 2013; Morice et al., 1992), a higher CRT threshold on subsequent testing may not necessarily indicate worsening reflexive cough function, but rather adaptation to the test.

The question of which cough reflex threshold more accurately represents an individual's true cough reflex threshold is debateable. It can be argued that, for the

first assessment, the role of expectation and cortical modulation may exert less influence on the individual's response. On the other hand, perhaps the second result more accurately represents the true reflex, as the individual reaches the point where they can (presumably) no longer over-ride the cortical modulation that includes the learning effect. Future work is needed to clarify this issue.

11.4. Limitations.

There are inherent limitations in applying results from a healthy, young population to a clinical population. In Study I, participants were frequently observed to be competitive about achieving a more successful suppression of cough during their second assessment, despite the fact that they were not told what their first SCT was. In our young, healthy participants the effect of expectation and adaptation to the test was greater than one dose above initial thresholds. It is unlikely that a clinical population would have the same motivation and cognitive ability. Perhaps then, the issue of test repeatability becomes less relevant.

Results from the present study offer no evidence of diurnal variation in cough reflex sensitivity in young, healthy individuals using citric acid CRT and the tidal breathing/facemask method. There is, however, an order effect irrespective of time of day, confirming that healthy participants are able to volitionally modulate their cough response. In the present study, this order effect was greater than one dose above initial thresholds. Translating these findings to clinical practice, clinicians should interpret the results of repeated cough reflex tests with caution.

11.5. Reducing Aspiration Pneumonia.

Based on prior research (Addington, Stephens, & Gilliland, 1999; Miles et al., 2013a; Wakasugi et al., 2008), it was hypothesized that the DiSP would guide clinicians towards making appropriate management decisions that would ultimately result in a

reduced rate of AP. This research confirms this hypothesis. Incorporating the DiSP into routine practice significantly improved patient outcomes. This study supports previous research documenting reduced AP rates when clear clinical decision-making pathways are in place for dysphagia screening (Hinchey et al., 2005; Lakshminarayan et al., 2010; Odderson et al., 1995; Yeh et al., 2011) and management (Addington, Stephens, & Gilliland, 1999; Burek et al., 2008; Odderson & McKenna, 1993). The effectiveness of such pathways may result from the elimination of bias imposed by clinical judgement as a contributing factor in patient outcomes. Similar research that used CRT but did not dictate subsequent management found no difference in AP rates or mortality (Miles et al., 2013a). When taken in the context of the study by Miles et al. (2013a), it can be inferred that the use of a protocol may be as important, or indeed more important, than the CRT itself in reducing the risk of AP in patients with acute stroke. The advantage of the DiSP protocol over most other published protocols is that it provides the clinician with information about patients' risk of silent aspiration and includes clear guidelines on the appropriate management of patients at high risk of, or identified as presenting, silent aspiration.

The DiSP accounted for only 8 % of the reduction in AP with individual patient factors accounting for an additional 9 %. The remaining 83 % of the variance was unexplained in the regression model. However, the model did correctly predict 84 % of pneumonia cases based on the DiSP. The DiSP was the only major change in medical practice that occurred since the Miles et al. (2013a) study, supporting the hypothesis that the results are attributable, in part, to this protocol.

Of note, more patients passed their initial CRT in the DiSP group (73 %) compared to the control group (61 %), suggesting that there may have been more 'silent aspirators' in the control group. It is concerning that clinicians in the control

group, despite being able to identify potential silent aspirators, had a low referral rate for VFSS at 46 %. This highlights the importance of a standardised approach to dysphagia assessment. The rates of CRT 'fails' in both the DiSP group (27 %) and control group (38 %) are higher than the 10 % fail rate reported by Addington et al. (1999) however, the difference in tussive agent and CRT administration used by Addington et al. may explain this difference in findings. Furthermore, Addington et al. do not describe how data from patients who could not achieve a complete lip seal or follow test instructions were handled. Other published reports where citric acid CRT was used report fail rates of 27 % among patients with acute stroke (Guillén-Solà et al., 2015) and 23 – 45 % among patients with mixed aetiologies (Miles et al., 2013b; Wakasugi et al., 2008, 2014), which are similar to the fail rates observed in the present study.

11.6. Reducing Mortality

Use of the DiSP did not result in decreased mortality or pneumonia-related mortality. Although not statistically significant, mortality rates were slightly higher in the DiSP group, which may be clinically significant. This was a concerning finding and reinforces the vulnerability of this patient population. It is unlikely that the higher mortality rates in the DiSP group were due to site-specific differences in medical practice, as study site did not emerge as a main effect in the regression model.

Examination of specific cases of pneumonia-related mortality in the DiSP patients revealed that the majority (63 %) had passed their initial CRT, with subsequent feeding management decisions made at the discretion of the SLT. It could be hypothesized that this group may represent patients with false negative results on the CRT who were inappropriately recommended an oral diet. This may also reflect weaknesses in clinical practice patterns. There was one case where a SLT did not

refer a patient for VFSS until the sixth review, despite noting overt signs of aspiration on five prior reviews. While the DiSP may be effective at identifying and managing patients with silent aspiration, future iterations of the DiSP should carefully consider ways to improve the management of patients who pass the CRT. Cases such as this highlight the need for SLTs to provide standardised management rather than relying on clinical judgement.

It is possible that other differences between the groups existed, such as a higher tendency for patients/families to request 'comfort cares' rather than aggressive medical intervention in the DiSP group, or the presence of other comorbidities that were not measured. Naughton et al. (2000) provide a model for predicting mortality in elderly patients with community-acquired pneumonia. Increased respiratory and pulse rate, altered mental status and co-existing dementia were all significant predictors of pneumonia-related mortality, with the probability of death greater than 30 % if two or more of these features are present (Naughton et al., 2000). Future studies should consider incorporating these diagnostic outcomes into regression analysis models for mortality.

11.7. Secondary Benefits of the DiSP

The findings from the present study suggest that the DiSP not only had a direct effect on AP but had secondary benefits for patients as well. It was hypothesized that patients in the DiSP group would have a significantly reduced length of hospital admission compared to patients in the control group. This hypothesis was accepted, with a reduction in acute length of stay from nine to six days and reduction in total hospitalisation from 32 to 24 days. This may be attributable to the lower incidence of AP in the DiSP group, resulting in a less complicated course of admission. Another possibility is that infections such as AP can

suppress neural recovery from stroke (Aslanyan et al., 2004). Pneumonia, in particular, can cause hypoxia and result in extension of the stroke (Aslanyan et al., 2004). Lower infection rates in the DiSP patients may have contributed towards their overall stroke recovery and resulted in a reduced length of hospitalisation.

Another secondary benefit from the DiSP that was not hypothesized was improved post-stroke independence, as evidenced by a greater proportion of DiSP patients living in their own home or a rest home at three months post-stroke compared to control patients, who were more likely to be in hospital. This is the first report of improved long-term benefits in a stroke population following the implementation of a dysphagia protocol. As mentioned above, this effect may have been due in part to lower rates of AP among DiSP patients resulting in improved stroke recovery and independence. Of course, domicile is but one facet of an individual's independence. Future research in this area may benefit from including a more standardised approach to measures of independence, such as the Functional Independence Measure™ (Guide for the Uniform Data Set for Medical Rehabilitation, 1996). Such an approach was not possible in the present study due to inconsistent documentation in patients' medical files but would have admittedly increased the validity of the findings.

It was also hypothesized that the DiSP would result in improvements in diet-level tolerance and route of intake. The specific hypothesis that significantly fewer DiSP patients would require an alternative route of intake compared to control patients could not be accepted; however, the low overall rates of alternative (i.e. tube) feeding across both the DiSP group (n = 1) and the control group (n = 3) were likely too low to reveal meaningful differences. The hypothesis that diet restriction at three months would differentiate DiSP patients from control patients was accepted. While 43 % of patients in the control group were on some form of modified diet, only 19 %

of DiSP patients continued to require diet modification. It is possible that the increased rate of VFS in the DiSP group meant that clinicians felt more confident about recommending higher (i.e. closer to normal) diet levels for these patients compared to clinicians in the control group, who relied more on clinical observation. The direct relationship between use of instrumental versus clinical assessment, clinician confidence and oral feeding outcomes has not been reported in the literature. Although quality of life was not included as an outcome measure in this study, there is evidence that patients generally do not enjoy texture-modified diets (Garcia, Chambers IV, & Molander, 2005; Ickenstein et al., 2010). It is reasonable to suggest that the DiSP, by means of improved diet level tolerance, may lead to improved quality of life among post-stroke patients. Further investigation is required to confirm this.

Not only do the results of the present study reflect positive functional outcomes for patients with acute stroke and dysphagia, in addition, the DiSP may benefit healthcare providers by reducing the costs associated with AP. Wilson (2012) reported that an additional $27,633 per patient was spent when pneumonia accompanied stroke. In the present study, no patients in the DiSP group were re-admitted to hospital with AP during the three-month follow-up period, although there may have been other contributing factors to this besides the DiSP.

The DiSP costs very little to implement, at less than $12 per test (cost of disposable face mask, tubing and sterile citric acid) plus a one-off cost of $400 per nebuliser. While there were increased costs associated with the increased rate of VFS, evidence from a recent cost-analysis model (Wilson & Howe, 2012) suggests that it is more cost-effective to refer every patient with acute stroke for a VFSS, compared to clinical examination alone or clinical examination followed by a VFSS. Clearly there

are ethical and resource limitations that prevent the implementation of VFS for every patient with acute stroke. The DiSP offers an alternative by identifying the patients who may benefit the most from VFS. A detailed cost-analysis study is needed to confirm the cost-benefit ratio of implementing the DiSP and is a suggested direction for future research.

11.8. Changes in Clinical Practice

Clinician adherence to the DiSP was encouragingly high at 90 %. Duncan et al. (2002) have reported wide variability of clinician compliance with stroke rehabilitation guidelines, from 18-92 % in acute rehabilitation settings. In the present study, clinicians were able to administer CRT and incorporate the protocol into clinical routine accurately and without the need for on-going support or training. Analysis of cases where the DiSP was not adhered to revealed a need for clarification around the use of compensatory strategies following VFSS. The DiSP does not currently include compensatory strategies which may contribute to confusion about appropriate management of some patients.

The high rate of clinician adherence to the DiSP suggests that, overall, clinicians accepted the protocol. However, there were some exceptions. Qualitative analysis of informal comments suggested that some clinicians did not agree with the 'one-size-fits-all' approach to dysphagia assessment. There were a few cases where patients with very mild stroke symptoms failed the CRT and were subsequently recommended nil-by-mouth and referred for a VFSS. Comments made by clinicians suggested that they found it difficult to justify this decision to patients and medical teams and did not necessarily agree with the decision themselves, preferring to proceed with a clinical evaluation of oral trials. Unfortunately, like any assessment of aspiration risk, the CRT does not have 100 % sensitivity and there is a trade-off

between identifying true positives and accepting the chance of also identifying false positives. Despite this, over-managing a small number of patients may be justified if the alternative is under-management of many patients, which can occur when clinicians rely solely on their own judgement. Without undertaking a formal, qualitative analysis of clinicians' views on the DiSP, it is difficult to comment on overall acceptability. Future work in this area should consider analysing clinicians' views on barriers and facilitators to incorporating the DiSP into clinical practice. This may provide insight into ways to increase adherence to the DiSP, which in turn may lead to further improvements in patient outcomes.

The results of the present study suggest a positive shift in clinical behaviour. Patients in the DiSP group were referred for instrumental swallowing assessment at a rate of 31 %, with 95 % of patients who failed the CRT referred for VFSS. This compares to reports of 25 % or less in the United Kingdom (Cocks & Ferreira, 2013), 36 % in Canada (Martino, Pron, & Diamant, 2004) and 60 % in the United States of America (Carnaby & Harenberg, 2013), although these reports come from a variety of clinical settings and patient populations. A recent study from New Zealand (Miles et al., 2013a) identified a referral rate of just 12 % among patients with acute stroke and dysphagia. In the study by Miles et al. (2013a), clinicians who did not use CRT frequently used pneumonia as a clinical indicator of dysphagia, referring for instrumental assessment only once pneumonia had developed. In the present study, a change in clinical decision-making was evident. Only five patients developed AP prior to being referred for an instrumental swallowing assessment, four of whom had presented with suspected or diagnosed pneumonia on admission to hospital.

There was a risk that the use of CRT may have led to clinician over-confidence in managing dysphagia at the bedside, if the results of a passed CRT were

over-interpreted as meaning the patient was not dysphagic. In fact, the opposite was observed, with referral rates for VFSS significantly increased compared to historical data (Miles et al., 2013a). Furthermore, 36 % of referrals for VFSS in the DiSP group were for patients who had passed their initial CRT. The significant decrease in AP rates observed in the present study is likely to be a reflection of the structured approach to dysphagia management rather than the use of CRT alone.

11.9. Managing Dysphagia by Withholding Oral Intake.

The issue of managing dysphagia by withholding oral intake requires further comment. International guidelines for the management of acute stroke (Jauch et al., 2013; Lindsay, Furie, Davis, Donnan, & Norrving, 2014; Stroke Foundation of New Zealand & New Zealand Guidelines Group, 2010) state that all patients with acute stroke should be *nil per orem* until their swallowing has been assessed and that this should occur within 24 hours of the stroke. The Royal College of Physicians (Gomes, Hookway, & Weekes, 2014) recommend that patients who are deemed unsafe for oral intake should be considered for enteral feeding within 24 hours. In the present study, patients' swallowing was assessed within 1 day (on average) of admission to hospital with many patients (64 %) resuming oral intake following this assessment. The use of the DiSP delayed the resumption of normal oral intake for only ten patients (3.5 %) for an average of two days (range = 1 – 3 days). The remaining patients were found to require diet modifications following VFSS. These figures may be considered acceptable when taken in the context of the overall reduction in AP that was observed following initiation of the DiSP. For the ten patients, this short-term period of discomfort was managed with enteral hydration and administration of medication via alternative routes where possible.

11.10. Future Directions.

Consistent with the discussion above, there are four issues that future research in this area should address. First, the use of a retrospective control group is admittedly problematic. This study design was unavoidable because the DiSP was already in place at the study location at the time this research was conducted, therefore no control group was available with the imposition of significant bias. However, a randomised controlled trial is needed to confirm this and is suggested for future research in this area.

Second, SLPs and nursing staff were relied upon to identify potential research participants. It is possible that some patients were not identified by accident or because staff felt that patients or their families would not want to participate in research due to the seriousness of their condition. Several patients were also excluded from the study as their medical team made decisions about oral intake that over-rode the DiSP. In most cases, this involved patients with very mild stroke symptoms. By not including these patients in the study, the sample is biased against patients with mild strokes. Future work should make efforts to recruit patients with a variety of stroke-related impairments.

Third, a criticism of the DiSP is that emphasis is placed on the function of a single cranial nerve (vagus), tested with the CRT. This test intends to identify patients with the potential for silent aspiration and subsequent AP. However, AP is, by nature, multifactorial and eliminating it will require a multi-faceted approach. Future work may consider expanding the DiSP to include the assessment and management of other known risk factors for AP, such as oral hygiene and mobility (Langmore et al., 1998; Langmore, Skarupski, Park, & Fries, 2002; Quagliarello et al., 2005; Terpenning et al., 2001).

Finally, including a survival curve analysis could also have been useful in revealing a critical window of time when patients with acute stroke were the most at risk for AP or identify a point during stroke recovery where the DiSP was the most effective in reducing AP rates. However, such an analysis requires detailed outcome measurement data that allows the researcher to pinpoint the day on which the infection presented. This was not possible in the present study due to the large variability in the documentation of patient symptoms and an absence of daily monitoring post-discharge from hospital, but is suggested for future work.

11.11. Risk Factors for Aspiration Pneumonia: Pathogenic Oral Bacteria.

The aspiration of pathogenic oral bacteria is another risk-factor for AP. Study III aimed to address this by investigating the relationship between pathogenic oral bacteria, cough reflex sensitivity and AP. Previous research (Watando et al., 2004) has suggested a potential relationship between oral bacteria levels and cough reflex sensitivity whereby reducing levels of oral bacteria through intensive oral hygiene was associated with improvements in reflexive cough sensitivity. However, the mechanism for such a relationship remains poorly understood. Patients with acute stroke and dysphagia are at risk of increased levels of pathogenic bacteria, poor cough reflex sensitivity and developing AP, yet the relationship between these variables remains undocumented.

In this study, it was hypothesized that relative levels of each of the target bacteria would increase from the point of admission to the point of discharge as participants may have been unable to perform their usual oral hygiene routines and relied on others to do this for them. Oral care is often overlooked in the context of acute medical or elderly care settings (Salamone, Yacoub, Mahoney, & Edward, 2013) due to time constraints (Wårdh, Hallberg, Berggren, Andersson, & Sörensen,

2000), oral hygiene being perceived as an undesirable task (Wårdh et al., 2000), lack of awareness/education/training around the importance of oral hygiene (Kuramoto et al., 2011; Salamone et al., 2013; Talbot, Brady, Furlanetto, Frenkel, & Williams, 2005; Wårdh et al., 2000), lack of formal oral hygiene protocols (Talbot et al., 2005) and patient non-compliance (Wårdh et al., 2000).

11.12. Prevalence of Pathogenic Oral Bacteria.

Relative levels of target bacteria did not significantly change over time and the null hypothesis was retained. This finding could be interpreted in several ways. Results support Zhu et al. (2008), who found that the oral carriage of coliforms did not significantly change over a six month period in patients with acute stroke. On the other hand, it is also possible that bacteria levels in the present study did increase over time, but that the study was insufficiently powered to detect this change [$1 - \beta = .09$], resulting in a Type II error. Previous research (Millns et al., 2003) has identified an increase in Gram-negative, anaerobic bacteria in the acute phase of stroke, with a decrease in bacteria levels associated with stroke recovery. In the present study, the proportion of patients with detectable oral pathogens also significantly decreased over time.

It is also possible that the participants in the present study do not truly represent all patients with acute stroke and dysphagia. This is evidenced by low recruitment rates – out of 154 patients who met the inclusion criteria for the study, 52 (34 %) declined to participate. The most frequently cited reason for declining was the patient was too unwell, therefore it is likely that the results of this study are biased towards "well" patients. An analysis of stroke-severity ratings to confirm this was not possible due to inconsistent reporting in participants' medical records, however, the observation that 54 % of participants were able to independently complete oral

hygiene routines at admission to hospital suggests that the majority of the study population had only a mild physical impairment.

Difficulties in obtaining saliva samples at the point of admission to hospital may also explain the lack of significant changes in relative bacteria levels. When a patient is admitted to hospital with an acute stroke, stabilization and medical treatment are prioritized, making it difficult to access patients for research and obtain true baseline measures. For this reason, it was decided that any bacterial measures obtained within the first 48 hours following admission would be considered 'admission' measurements. The inherent flaw with this approach is that bacteria numbers increase exponentially over time in the absence of routine oral hygiene (Wilson, 2005). It is unlikely that the 'admission' measurements reported in the present study are a true reflection of patients' actual bacteria levels at admission to hospital. Future research of this nature could investigate other options for expedited recruitment and outcome measurement, such as having a researcher present in the emergency/acute assessment unit or collaborating with medical staff to obtain saliva samples at the point of admission and seeking patient consent to use the samples for research *post hoc*.

When attempting to compare bacteria levels at admission to bacteria levels at discharge from hospital, an unexpected difficulty was the wide variability in the length of hospitalization between participants. Some participants were admitted to and discharged from hospital on the same day, while others spent up to sixteen days on an acute stroke ward before being transferred to a rehabilitation ward. Collecting the second outcome measure after a certain number of days is one way to control for this variability, however this introduces a confounding variable of the participants' location. In the present study, every attempt was made to collect the second saliva

sample whilst participants' were still in hospital. It is possible that the variation in length of hospitilisation made it difficult to detect any differences in bacteria levels over time that may have been present.

Another potential confounding variable when attempting to measure relative bacteria levels is the time the sample is taken. Due to the busy nature of an acute hospital ward and difficulties accessing participants, time of sampling was not able to be controlled for in the present study. However, time of testing was included in the regression analyses and did not emerge as a significant predictor of relative bacteria levels. As bacteria levels tend to fluctuate throughout the day, it is also possible that taking only three samples over a one-month period was an insufficient number to draw accurate conclusions about the changes in bacteria levels over time. Ewan et al. (Ewan et al., 2015) obtained five bacterial samples from elderly hospitalized patients and identified significant trends in bacterial prevalence. Bacteria can be transient colonizers and, in Study III, may not have been present on the days that sampling took place. Future research may benefit from increasing the number of samples, however this would also increase analysis costs.

Alternatively, the results of the present study may be interpreted as evidence that patients on specialist acute stroke units are receiving adequate oral hygiene. Supporting this argument, the prevalence of *S. pneumoniae* in the present study was 17-25 %, compared with a 29 % prevalence in a similar study of elderly, hospitalized patients with lower limb fractures (Ewan et al., 2015), the prevalence of *S. aureus* was 1-2 % compared to 4-5 % reported elsewhere (Bousbia et al., 2012; Ewan et al., 2015) the prevalence of *E. coli* was 1 % compared to 8 % reported by Bousbia et al. (2012), the prevalence of *Streptococcus* was 38-57 % - lower than the 93 % prevalence previously reported in healthy participants (Rozkiewicz et al., 2006) and

the prevalence of *P. aeruginosa* was 5-10 %, lower than the 36 % prevalence in patients with post-stroke dysphagia reported by Hirota et al. (2010). On the other hand, *P. aeruginosa, K. pneumoniae* and *E. coli* are not typically found in healthy mouths, yet were detected in the present study within 48 hours of admission to hospital (*P. aeruginosa* and *E. coli*) and at discharge from hospital (*K. pneumoniae*). This finding suggests a 48-hour critical window may be present for preventing the colonization of potential respiratory pathogens in this patient population. Further comment on the adequacy of oral hygiene in the acute care setting is not possible, as the study was not designed to investigate this. Future research would benefit from the inclusion of control participants in order to draw clear conclusions on this topic.

Of course, antibiotic use is a major confounding variable when attempting to draw comparisons between data from hospitalized patients and normative data as well as investigate relationships between bacteria levels and variables such as cough reflex sensitivity and AP. Unfortunately, in the present study, detailed information about patients' antibiotic use was not available from medical records. Antibiotic use is the most likely explanation for the low observed rate of *Streptococcus* in the present study, as this species is highly prevalent amongst healthy individuals (Rozkiewicz et al., 2006). Watando et al. (2004) do not comment about whether antibiotic use was controlled for in their study of rest home residents and it is possible that some of their participants were also taking antibiotics. Future work in this area should consider excluding participants who are taking antibiotics. This would allow more accurate comparisons to normative data to be drawn.

The outcome measurement tools used in the present study require further comment. Previous research (Ewan et al., 2009) has indicated that for bacteria such as *S. aureus* and *P. aeruginosa*, sampling via oral rinse is the preferred method. This was

not possible in the present study due to the risk of aspiration in this cohort. Protected specimen brush sampling has also been suggested as a valid and reliable means of measuring respiratory pathogens (Mertens et al., 1998) however, this technique is not routinely conducted in New Zealand and was considered too invasive for the nature of this study. Oral swabs, while appealing due to their low level of invasiveness, patient acceptability and ease of use, are limited in their ability to collect saliva in patients with xerostomia, a condition not uncommon in the acute stroke population (Sellars et al., 2007). Although the use of nasopharyngeal swabs may have increased the yield of *S. pneumoniae* (Lieberman et al., 2006), sampling of oral bacteria was chosen because of the causative links between oral bacteria and AP (Bousbia et al., 2012; Simmons-Trau et al., 2004; Watando et al., 2004; Yoneyama et al., 2002; Yoshino et al., 2001). It is possible that the use of oral swabs resulted in lower bacteria yields compared to other methods. The data presented here represent the best measurements that could be taken without invasive sampling.

The present study measured bacterial outcomes in terms of relative levels rather than number of bacteria as it is not possible to convert directly from the amount of gDNA on a standard curve to a bacterial count (Siddiqi, Milne, Cullinan, & Seymour, 2014). Furthermore, measuring bacterial cells has little relevance in the present study due to a lack of published reports describing clinically relevant levels of bacteria. What constitutes a 'healthy' quantity of bacteria is yet to be documented and is necessary in order to draw comparisons between the oral bacteria in a person who has AP. Attempting to define clinically relevant bacteria levels was not possible in the present study due to the relatively low incidence of AP (10 %) and the limited number of repeated measures collected. However, among the participants who developed AP, 36 % had relative levels of target bacteria at admission that were higher than the

average admission levels of participants who did not develop AP. This trend warrants further investigation as findings may have direct implications for AP-risk screening at admission to hospital.

As the nature of this work was exploratory, it was decided to include a *Streptococcus* primer set rather than a *S. mitis*-specific primer set. Oral *Streptococcus* are found in abundance in the mouths of healthy adults (Malhotra-Kumar et al., 2004; Morita et al., 2004; Rozkiewicz et al., 2006) so were considered as a positive control in the present study and confirmation that the qPCR assays were functioning correctly. Due to *S. mitis* being the most dominant species of the oral *Streptococcus* (Malhotra-Kumar et al., 2004; Morita et al., 2004), evidence of amplification on qPCR can be considered as evidence of *S. mitis.* Of course, amplification of other *Streptococcus* spp cannot be ruled out and future work in this area should consider including a *S. mitis*-specific primer set to increase confidence in the interpretation of qPCR results.

11.13. The Relationship Between Oral Bacteria and Aspiration Pneumonia.

In contrast with a recent study which found a relationship between oral colonisation of *E. coli/P. aeruginosa/S. aureus/Methicillin-resistant S. aureus* (MRSA) and hospital-acquired pneumonia in elderly patients with lower limb fractures (Ewan et al., 2015), in the present study, AP was not associated with any bacterial variables. The main differences between these two studies are that Ewan et al. undertook bacterial sampling at five points during hospitalisation and measured hospital-acquired pneumonia rates over a 90-day period, compared to the present study which undertook bacterial sampling at three points in time and measured aspiration-pneumonia rates over a 30-day period. Ewan et al. report that 50 % of cases of pneumonia occurred after 25 days. Due to fluctuations in bacteria levels, increasing

the number of repeated measures of bacteria is likely to increase the chances of detecting transient colonisers. This in turn may reveal the relationship of these organisms to AP. For example, in the present study, the incidence of AP in those with *S. aureus* (n = 2) was 50 %; for those with *P. aeruginosa* (n = 20) the incidence was 15 % and a single participant with *K. pneumoniae* developed AP (100 %). However, these figures are drawn from such low base numbers that it is difficult to draw firm associations.

The prevalence of *E. coli* and *S. aureus* was slightly higher in the Ewan et al. study than the present study (2 % and 5 % versus 1 % and 2 %, respectively). However, the present study had a higher prevalence of *P. aeruginosa* at 5-10 % compared to 4 % reported by Ewan et al. Ewan et al.'s findings indicate that the presence of pathogenic oral bacteria is a significant factor in the development of hospital-acquired pneumonia in elderly patients with fractures. For patients with acute stroke, however, the pathogenesis of AP is more complex, involving other variables such as dysphagia severity and reflexive cough strength, which were not measured in the present study.

The one-month mortality rate in the present study was 9 %, however, as noted above, the study was biased towards 'well' patients. Of those patients who died, pneumonia was the leading cause of mortality, accounting for 56 % of deaths. It is clear that interventions to prevent pneumonia in this population are warranted.

Any relationship between bacteria and cough sensitivity was not apparent using clinically-relevant doses of citric acid. However, this relationship has previously been reported using lower doses of citric acid (Watando et al., 2004) and is worth investigating further. The present pilot study was insufficiently powered to detect this relationship, which may be due in part to power calculations that were

based on a higher reported rate of AP (Miles et al., 2013a) than was observed. However, given the difficulties recruiting participants, the prolonged follow-up period and the time needed to develop qPCR assays, it was not possible to recruit more participants. This pilot study adds to the limited amount of literature describing clinical, microbiological, and pneumonia-related outcomes and will form the basis for future, large-scale studies investigating possible relationships between these variables.

Measuring AP is difficult because there is no gold standard diagnostic test. The present study relied on previously published diagnostic criteria (Mann et al., 1999), applied to documentation from physicians and nursing staff in participants' medical files. This method of outcome measurement is limited by the accuracy of the documentation as well as the subjective observations of medical staff, for example, in observing for chest crepitations and crackles.

11.14. Cough Reflex Sensitivity in Patients with Acute Stroke.

Overall cough reflex sensitivity levels in patients with acute stroke did not change over time. This was an unexpected finding, given that patients were all in the acute stages of stroke recovery. Addington et al. (2005) described a temporary or permanent suppression of the reflexive cough response in the acute stages of stroke which they termed "brainstem shock". This phenomenon was only observed in 12 % of the participants in the present study, who did not trigger a reflexive cough at the highest level of citric acid (1.2 mol/L) upon admission to hospital. Indeed, the vast majority of participants (70 %) had cough reflex sensitivity levels of 0.4 mol/L or less, which is within the normal range for elderly people (Monroe et al., 2014). This finding supports that the present study was biased towards 'well' patients.

As mentioned above, due to difficulties accessing patients immediately following their admission to hospital, the window of opportunity to measure patients'

cough reflex sensitivity levels at their most impaired may have been lost. Future research in this area should consider ways to improve access to acute patients.

It is also possible that a Type II error was introduced, as suggested by the low observed power [$1 - \beta = .08$]. Another explanation for the lack of observed change in cough reflex sensitivity levels is the possibility of a flooring effect. The lowest dose of citric acid that was administered to participants was 0.4 mol/L. This dose was selected based on the average suppressed cough threshold level of healthy elderly people (Monroe et al., 2014). However, significant changes in cough reflex sensitivity levels over time have been documented in healthy elderly subjects using lower doses of citric acid. Watando et al. (2004) reported that participants' cough reflex thresholds changed from approximately 0.15 mol/L to 0.08 mol/L over a 30-day period when provided with intensive, daily oral hygiene. It is possible that similar changes may have been observed in the present study if lower doses of citric acid were used.

Gender emerged as a significant predictor of cough reflex thresholds upon admission to hospital, with being female associated with higher cough reflex thresholds. This is in contrast to previous reports that females have lower cough reflex thresholds compared to males (Monroe et al., 2014; Rostami-Hodjegan et al., 2001). Given that this finding was not observed at discharge from hospital or at one month post-stroke, it is likely to be an incidental finding rather than a true gender effect.

Cough reflex sensitivity was measured by a single researcher (S.D.) on the basis of a subjective rating of cough presence/absence. The CRT method used in the present study was selected due to its portability and ease of use in an acute hospital setting, however, future work in this area may benefit from the use of objective measures of reflexive cough, such as cough expiratory volume and peak expiratory flow rate, as well as the inclusion reliability measures.

Chapter 12. Concluding Remarks

Aspiration pneumonia is a serious, yet largely preventable, complication of acute stroke. There is a growing body of evidence supporting the use of CRT in clinical dysphagia assessment. As CRT becomes more widely used, there is a need for standardisation of CRT methodology. This research does not support the need to control for time of testing, however, has identified that adaptation to the test may represent a potential confounding variable. Until a standard CRT method is accepted, clinicians and researchers should refer to the existing literature with close attention to methodology.

This research contributes to the literature by providing evidence that an assessment and management protocol may be as important as the CRT itself in preventing AP. When CRT is used in the context of a protocol, the rate of AP in acute post-stroke patients significantly decreases. The DiSP protocol is appropriate for use in acute medical settings and requires little training or cost to implement. The addition of this protocol into clinical routine is endorsed.

This research was not able to convincingly confirm or deny that a relationship exists between cough reflex sensitivity and oral bacterial levels, but has identified methodological limitations and established directions for future work in this area. If future research identifies a strong relationship between oral bacteria and cough reflex sensitivity, there would be an ideal opportunity for establishing oral hygiene interventions, which may further reduce rates of AP.

AP remains an issue for stroke patients in New Zealand and internationally. Further research is needed to refine dysphagia protocols and understand the effects of oral bacteria on reflexive airway protection mechanisms. This will ultimately inform

changes in clinical practice that will reduce the risk of AP in vulnerable patient populations.

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Appendix I: Information Sheets and Consent Forms

Participant Information Sheet

Study title:	**Consistency of the reflexive cough response in healthy young adults**		
Locality:	**University of Canterbury Human Ethics Committee**	Ethics committee ref.:	**HEC 2014/36**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 378 6068**

You are invited to take part in a research project about the cough reflex in healthy people. Taking part is your choice. If you don't want to take part, you don't have to give a reason. If you want to take part now, but change your mind later, you can pull out of the study at any time.

This information sheet will help you decide if you'd like to take part. It sets out why we are doing the study and what is involved. It also explains the risks and benefits, and what happens after the study. We will go through this with you and answer your questions. This will take about 5 minutes. You may also want to talk about the study with family, whānau, friends, or healthcare members. Feel free to do this.

If you want to take part, you will need to sign a consent form. You will be given a copy of this information sheet and consent form to keep.

This document is 3 pages long. Please make sure you have all the pages.

This study is being run by Sarah Davies with supervision from Dr Maggie-Lee Huckabee (University of Canterbury) and Dr John Fink (Canterbury District Health Board). For more information, please contact the researchers during work hours. Their numbers are written at the end of this document.

If you need an interpreter, this can be arranged.

1. Why are we doing the study?

The cough reflex test is a commonly used assessment for people who have swallowing disorders (known as dysphagia). It is also often used in swallowing research. We want to know whether a person's response to cough reflex testing is consistent over time. The results from this study will help us learn more about how we can best use cough reflex testing with patients, and in research.

2. What is involved?

If you agree to enrol in the study, the following will occur:

1. You will be asked to sign a consent form.
2. You will be asked to complete a short questionnaire. The questionnaire will help us to make sure that you are eligible to take part in this study.
3. You will be given a new toothbrush, and taken to a room with a basin. You will be asked to brush your teeth for two minutes and then rinse your mouth with water twice.
4. The researcher will then test your cough reflex:
    a. A face mask will cover your nose and mouth. The face mask is attached to a device that turns liquid into mist, called a nebuliser.
    b. The nebuliser will turn small amounts of citric acid or saline into mist. You will be asked to quietly breathe the mist for 15 seconds.
    c. The mist may make you want to cough. Try not to cough.
5. During the cough reflex test, a video camera will be used to record your response. The video will be viewed by the researcher, and one other researcher at the Swallowing Rehabilitation Research Lab only. The video will be used to help us check our measurements, and will then be erased.
6. Steps 2-5 will be repeated once more, at a separate time.
7. You will not have to do anything else.

3. What are the possible benefits and risks?

There is no benefit to you from doing this study.

There are no known adverse side effects of cough reflex testing. The test is administered by a trained researcher.

4. What would happen if you were injured in the study?

If you were injured in this study, which is unlikely, you could apply for compensation from ACC just like if you were injured in an accident at work or home.

5. What are your rights?

Taking part in this study is your choice. If you don't want to take part, you don't have to give a reason. If you want to take part now, but change your mind later, you can pull out of the study at any time.

You have the right to see your personal information collected during this study at any time. If, during the study, new information about the risks and benefits come to light, you will be told of this.

The information we collect will be kept confidential by using a number instead of your name. All information will be stored in a locked cabinet at the New Zealand Brain Research Institute (NZBRI). Electronic data will be stored on a password-protected computer at the NZBRI.

6. What will happen after the study ends, or if you pull out?

The data we collect will be kept securely at the NZBRI for 10 years. After this, it will be shredded under the supervision of either Sarah Davies or Maggie-Lee Huckabee.

Findings from this study will be prepared for publication in international journals. Results may be presented at national and/or international conferences. Findings will also form the basis of a doctoral thesis. A thesis is a public document, and can be viewed at the University of Canterbury library.

If you want, you can have a copy of the publications that arise from this research. Please be aware that a substantial delay may occur between data collection and completing the final report.

7. Where can you go for more information, or to raise concerns or complaints?

If you have any questions about the study at any stage, please contact:

Sarah Davies, Ph.D. candidate
(03) 378 6068
sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee, Senior researcher
(03) 378 6070
maggie-lee.huckabee@canterbury.ac.nz

This project has been reviewed and approved by the University of Canterbury Human Ethics Committee, and participants should address any complaints to:

The Chair, Human Ethics Committee

University of Canterbury, Private Bag 4800, Christchurch

human-ethics@canterbury.ac.nz

Participant Consent Form

Study title:	**Consistency of the reflexive cough response in healthy young adults**		
Locality:	**University of Canterbury Human Ethics Committee**	Ethics committee ref.:	**HEC2014/36**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

Declaration by participant:

I have read (or have had read to me in my first language) and I understand the Participant Information Sheet. I have had the opportunity to ask questions. I am satisfied with the answers I have received.

I freely agree to participate in this study.

I have been given a copy of the Participant Information Sheet and Consent Form to keep.

I understand that a video recording will be made, which will be viewed by the primary researcher and one other researcher at the NZBRI. The video file will then be erased.

☐ I would like to receive a copy of the results of this study

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant. I have answered their questions. I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature:	Date:

Participant Information Sheet

Study title:	**Can a clinical care protocol reduce pneumonia rates in patients with acute stroke?**		
Locality:	**Northern A**	Ethics committee ref.:	**13/NTA/111**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

You are invited to take part in a research project about swallowing care in patients with stroke. Taking part is your choice. If you don't want to take part, you don't have to give a reason. It won't change the care you receive. If you want to take part now, but change your mind later, you can pull out of the study at any time.

This information sheet will help you decide if you'd like to take part. It sets out why we are doing the study and what is involved. It also explains the risks and benefits, and what will happen after the study. We will go through this with you and answer your questions. This will take about 5 minutes. You may also want to talk about the study with family, whānau, friends, or healthcare members. Feel free to do this.

If you want to take part, you will need to sign a consent form. You will be given a copy of this information sheet and consent form to keep.

This document is 5 pages long. Please make sure you have all the pages.

This study is being run by Sarah Davies with supervision from Dr Maggie-Lee Huckabee (University of Canterbury), Dr John Fink and Dr Geoffrey Tompkins (University of Otago). The University of Canterbury and the Canterbury District Health Board are collaborating in this study. This study has been approved by the University of Canterbury Human Ethics Committee and The New Zealand Health and Disability Ethics Committee.

For more information, you can contact the researchers during work hours. Their numbers are written at the end of this document.

If you need an interpreter, this can be arranged.

If you have any queries or concerns about your rights in this study, contact a Health and Disability Advocate:

South Island: 0800 377 766 or (03) 377 7501 in Christchurch.
Email: advocacy@hdc.org.nz

8. Why are we doing the study?

In many patients with stroke, the ability to swallow changes. These patients are at risk of getting chest infections. We want to know whether the way we manage these patients is effective at reducing chest infections. The results from this study will help improve our care of these 'at risk' patients.

9. What is involved?

Being involved in this study will not change your care. You will receive the same care regardless of whether you enrol in this study.

If you agree to enrol in the study, the following will occur:

1. You will be asked to sign a consent form.
2. Your speech-language therapist will assess your swallowing. This assessment is part of standard hospital care after stroke. It is not influenced by your involvement in this study.
3. Your speech-language therapist will use this information to plan your care. You will not need to do anything else.
4. When you leave the acute stroke ward, a researcher will review your medical file. She will check to see:
    a. If there were any problems with your swallowing
    b. If you had any formal tests of your swallowing
    c. Which foods and drinks were given to you
    d. If you were given antibiotics for a chest infection
5. After 3 months, she will review your medical file for the final time. She may phone your general practitioner. She will check whether you have had any problems with your swallowing. She will also check if you have been given antibiotics for a chest infection, or if you have returned to hospital.

10. What are the possible benefits and risks?

There is no benefit to you from involvement in this study. Your care will be the same whether or not you enrol in this study.

There are no known adverse side effects of any part of the standard bedside swallowing assessment. The assessment is performed by a trained speech-language therapist who will monitor you for adverse signs.

11. What would happen if you were injured in the study?

If you were injured in this study, which is unlikely, you could apply for compensation from ACC just like if you were injured in an accident at work or home.

12. What are your rights?

Taking part in this study is your choice. If you don't want to take part, you don't have to give a reason. It won't affect your care. If you want to take part now, but change your mind later, you can pull out of the study at any time.

You have the right to see your personal information collected during this study at any time. If, during the study, new information about the risks and benefits to you come to light, you will be told of this.

Your medical information will be kept confidential by using a number instead of your name. All information will be stored in a locked cabinet at the Rose Centre for Stroke Recovery & Research at St George's Medical Centre. Electronic data will be stored on a password-protected computer at the Rose Centre.

13. What will happen after the study ends, or if you pull out?

The data we collect will be kept securely at the Rose Centre for 10 years. After this, it will be shredded under the supervision of either Sarah Davies or Maggie-Lee Huckabee.

Findings from this study will be prepared for publication in national and/or international journals. Results may be presented at national and/or international conferences. Findings will also form the basis of a doctoral thesis. A thesis is a public document, and can be viewed through the library at the University of Canterbury.

If you want, you can have a copy of the publications that arise from this research. Please be aware that a substantial delay may occur between data collection and completing the final report. Alternatively, you can choose to have findings discussed with you personally by the lead investigator.

14. Where can you go for more information, or to raise concerns or complaints?

If you have any questions, concerns or complaints about the study at any stage, please contact:

Sarah Davies, Ph.D. candidate
(03) 364 2307
sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee, Senior researcher
(03) 364 2014
maggie-lee.huckabee@canterbury.ac.nz

If you want to talk to someone who isn't involved with the study, contact an independent health and disability advocate:

Phone: 0800 555 050
Email: advocacy@hdc.org.nz

You can also contact the ethics committee that reviewed and approved this study:

Phone: 0800 4 ETHICS

Email: hdecs@moh.govt.nz

Participant Consent Form

Study title:	**Can a clinical care protocol reduce pneumonia rates in patients with acute stroke?**		
Locality:	**Northern A**	Ethics committee ref.:	**13/NTA/111**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

Declaration by participant:

I have read (or have had read to me in my first language) and I understand the Participant Information Sheet. I have had the opportunity to ask questions. I am satisfied with the answers I have received.

I freely agree to participate in this study.

I have been given a copy of the Participant Information Sheet and Consent Form to keep.

☐ I would like to receive a copy of the results of this study

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant. I have answered their questions.

I believe that the participant understands the study and has given informed consent to participate.

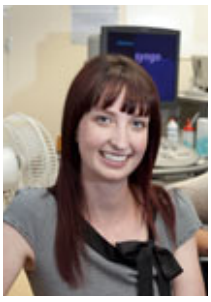
Researcher's name:

Signature:	Date:

Information Sheet for Participants with Aphasia

Research into swallowing in patients with stroke

Researchers:



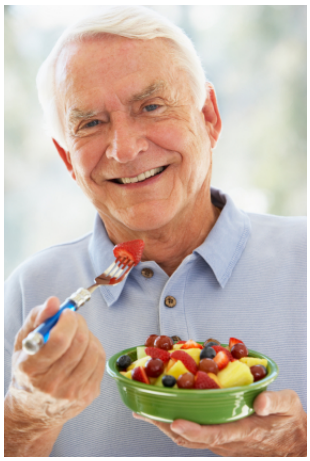
Sarah Davies
PhD student
University of Canterbury

Dr Maggie-Lee Huckabee
Senior researcher
University of Canterbury

Dr John Fink
Consultant neurologist
Canterbury District Health Board

Dr Geoffrey Tompkins
Senior researcher
University of Otago

We are researching **swallowing** in patients with **stroke**.



Taking part in this study is **your choice**.

my choice

You may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers.

If you agree to take part in this study, you will be asked to **sign a consent form**.

1. Why are we doing the study?

In many patients with **stroke**, the ability to **swallow** safely is affected.

These patients may get a **chest infection**.

→

We want to **learn** how we can **stop chest infections**.

How?

This study will help to improve the **care** of patients with **stroke**.

2. What would I have to do?

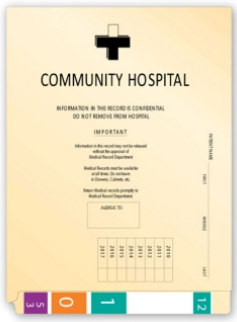
If you agree to take part in this study, you will need to **sign a consent form**.

Your speech-language therapist will then **test your swallowing** as she would normally do.

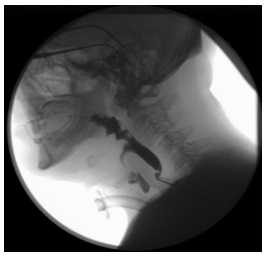
This test is part of **normal hospital care after stroke.** Taking part in this study will not change this.

You **do not need to do anything** else.

When you leave the acute stroke ward, a **researcher** will **look at your medical record** to see:



- If you had any other **tests** of your swallowing



?

- Which **foods** and **drinks** you were given

?

- If you had a **chest infection**

?

The researcher may also phone your GP.

3. What are the risks and benefits?

This will **help researchers** learn more about swallowing treatment.

This may help people who have a stroke.

There is **no benefit** to you.

There are **no risks** to you.

Being involved in this study will **not change your treatment**.

You will receive the **same treatment** whether you take part in this study, or not.

This is **not therapy**.

4. What would happen if I was injured in the study?

If you were **injured** in this study, which is **unlikely**, you could apply for compensation from **ACC** just like if you were injured in an accident at work or at home.

5. What are my rights?

Taking part in this study is **your choice**.

If you **do not want to** take part, you do not have to give a reason**.**
It won't change your care.

If you want to take part now, but **change your mind** later, you can **stop** at any time.

You have the right to **see your personal information** at any time.

If there is **new information** about the risks and benefits, we will **tell you**.

6. What will happen to my personal information?

All information is **confidential**. We will use a number instead of your name.

The information will be **locked** in the Department of Speech and Language therapy at Christchurch Public Hospital or The Princess Margaret Hospital.

Some information will be kept on a **password**-protected **computer** at the New Zealand Brain Research Institute.

At the end of this study, the information will be **kept safely** at the New Zealand Brain Research Institute for **10 years**. Then it will be **destroyed**.

10

years

→

Findings from this study may be presented:

- In scientific journals
- At scientific meetings
- In a doctoral thesis, which the **public** can read at the University of Canterbury library

If you want, **you can have a copy** of the findings. This may take some time to prepare.

If you want, the main researcher will tell you about the findings.

7. Where can I go for more information about the study?

If you have any **questions** about the study at any stage, please contact:

Sarah Davies

Ph.D. candidate

(03) 378 6068

sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee

Senior researcher

(03) 378 6070

maggie-lee.huckabee@canterbury.ac.nz

8. Where can I go to raise concerns or complaints about the study?

If you want to **talk** to someone who is not part of the study, you can contact a **Health and Disability Advocate**:

Phone:  0800 555 050

Email:  advocacy@hdc.org.nz

If you have any **queries** or **concerns** about your **rights** as a participant in this study, you can contact a **Health and Disability Advocate**:

 Christchurch: (03) 377 7501

 South Island: 0800 377 766

 Email: advocacy@hdc.org.nz

This research has been **approved** by the Human Ethics Committees of The University of Canterbury and The New Zealand Health and Disability Ethics Committee.

You can **contact** the **ethics committee** that approved this study on:

Phone: 0800 4 ETHICS

Email: hdecs@moh.govt.nz

Consent Form for Participants with Aphasia

Study title:	**Can a clinical care protocol reduce pneumonia rates in patients with acute stroke?** ("Research into swallowing in patients with stroke")		
Locality:	**Northern A**	Ethics committee ref.:	**13/NTA/111**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 378 6068**

I understand:

- I do not have to take part. It is **my choice.**
- I can **change my mind.**

- I can **ask questions at any time.**

- I can **stop** the research at **any time.**

- There is **no danger** in doing this research.

I understand what will happen:

- A speech-language therapist will **test my swallowing** as would **normally** happen.

- A **researcher** will get information from **my medical records**.

I understand the benefits of this research:

- This will **help researchers** learn about swallowing care.
- This may **help people** who have a **stroke**.
- This will **not** benefit me.
- This is **not therapy**.

I understand that my personal information:

- Will be kept **safely**.
- Will be **confidential**.
- Will be destroyed after **10 years**.

☐ I **understand** this research.

☐ I have had the chance to **ask questions** and I am happy with the answers.

☐ I **agree** to participate in this study.

☐ I have been given a **copy** of the Participant **Information** Sheet and Consent Form to keep.

☐ I would like to see a **copy of the results** of this study

Participant's name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

STATEMENT BY RELATIVE/FRIEND/WHANAU

Lay title:	Can a clinical care protocol reduce pneumonia rates in patients with acute stroke?
Principal investigator:	Sarah Davies
Participant's name:	

I have read and I understand the information sheet dated 13.05.13 for people taking part in the study designed to evaluate the management of swallowing for patients who have had a stroke. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

I believe that _____ (participant's name) would have chosen and consented to participate in this study if he/she had been able to understand the information that I have received and understood.

I understand that taking part in this study is voluntary and that my relative/friend may withdraw from the study at any time if he/she wishes. This will not affect his/her continuing health care.

I understand that his/her participation in this study is confidential and that no material which could identify him/her will be used in any reports on this study.

I know whom to contact if anything occurs which I think he/she would consider a reason to withdraw from the study.

This study has been given ethical approval by the Northern A Regional Health & Disability Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.

I/my relative/friend would like a copy of the results of the study.	☐ Yes	☐ No
I believe my relative/friend would agree to his/her GP being informed of his/her participation in this study.	☐ Yes	☐ No

Signed:		Date:	
Printed name:			
Relationship to participant:			
Address for results:			

STATEMENT BY INDEPENDENT CLINICIAN

I confirm that participation in the study is not adverse to _____

(participant)'s interests.

Signed: | Date:

(Clinician)

Printed name:

STATEMENT BY PRINCIPAL INVESTIGATOR

I _____ *(name of investigator)* declare that this study is in the potential health interest of the group of patients of which _____ *(name of participant)* is a member and that participation in this study is not adverse to _____ *(name of participant)*'s interests.

I confirm that if the participant becomes competent to make an informed choice and give an informed consent, full information will be given to him/her as soon as possible, and his/her participation will be explained. If the participant makes an informed choice to continue in the study, written consent will be requested and if the participant does not wish to continue in the study, he/she will be withdrawn.

Signed: | Date:

(Principal Investigator)

(IF APPLICABLE AT A LATER STAGE)

I _____ *(participant)* having been fully informed about this study agree to continue taking part in it.

Signed: | | Date: | |

(Participant)

Participant Information Sheet

Study title:	**Can a clinical care protocol reduce pneumonia rates in patients with acute stroke?**		
Locality:	**Northern A**	Ethics committee ref.:	**13/NTA/111**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

You are invited to take part in a research project about swallowing care in patients with stroke. Taking part is your choice. If you don't want to take part, you don't have to give a reason. It won't change the care you receive. If you want to take part now, but change your mind later, you can pull out of the study at any time.

This information sheet will help you decide if you'd like to take part. It sets out why we are doing the study and what is involved. It also explains the risks and benefits, and what will happen after the study. We will go through this with you and answer your questions. This will take about 5 minutes. You may also want to talk about the study with family, whānau, friends, or healthcare members. Feel free to do this.

If you want to take part, you will need to sign a consent form. You will be given a copy of this information sheet and consent form to keep.

This document is 5 pages long. Please make sure you have all the pages.

This study is being run by Sarah Davies with supervision from Dr Maggie-Lee Huckabee (University of Canterbury), Dr John Fink and Dr Geoffrey Tompkins (University of Otago). The University of Canterbury and the Canterbury District Health Board are collaborating in this study. This study has been approved by the University of Canterbury Human Ethics Committee and The New Zealand Health and Disability Ethics Committee.

For more information, you can contact the researchers during work hours. Their numbers are written at the end of this document.

If you need an interpreter, this can be arranged.

If you have any queries or concerns about your rights in this study, contact a Health and Disability Advocate:

South Island: 0800 377 766 or (03) 377 7501 in Christchurch.
Email: advocacy@hdc.org.nz

15. Why are we doing the study?
In many patients with stroke, the ability to swallow changes. These patients are at risk of getting chest infections. We want to know whether the way we manage these patients is effective at reducing chest infections. The results from this study will help improve our care of these 'at risk' patients.

16. What is involved?
Being involved in this study will not change your care. You will receive the same care regardless of whether you enrol in this study.

If you agree to enrol in the study, the following will occur:

1. You will be asked to sign a consent form.
2. Your speech-language therapist will assess your swallowing. This assessment is part of standard hospital care after stroke. It is not influenced by your involvement in this study.
3. Your speech-language therapist will use this information to plan your care. You will not need to do anything else.
4. When you leave the acute stroke ward, a researcher will review your medical file. She will check to see:
    a. If there were any problems with your swallowing
    b. If you had any formal tests of your swallowing
    c. Which foods and drinks were given to you
    d. If you were given antibiotics for a chest infection
5. After 3 months, she will review your medical file for the final time. She may phone your general practitioner. She will check whether you have had any problems with your swallowing. She will also check if you have been given antibiotics for a chest infection, or if you have returned to hospital.

17. What are the possible benefits and risks?
There is no benefit to you from involvement in this study. Your care will be the same whether or not you enrol in this study.

There are no known adverse side effects of any part of the standard bedside swallowing assessment. The assessment is performed by a trained speech-language therapist who will monitor you for adverse signs.

What would happen if you were injured in the study?
If you were injured in this study, which is unlikely, you could apply for compensation from ACC just like if you were injured in an accident at work or home.

18. What are your rights?

Taking part in this study is your choice. If you don't want to take part, you don't have to give a reason. It won't affect your care. If you want to take part now, but change your mind later, you can pull out of the study at any time.

You have the right to see your personal information collected during this study at any time. If, during the study, new information about the risks and benefits to you come to light, you will be told of this.

Your medical information will be kept confidential by using a number instead of your name. All information will be stored in a locked cabinet at the Rose Centre for Stroke Recovery & Research at St George's Medical Centre. Electronic data will be stored on a password-protected computer at the Rose Centre.

19. What will happen after the study ends, or if you pull out?

The data we collect will be kept securely at the Rose Centre for 10 years. After this, it will be shredded under the supervision of either Sarah Davies or Maggie-Lee Huckabee.

Findings from this study will be prepared for publication in national and/or international journals. Results may be presented at national and/or international conferences. Findings will also form the basis of a doctoral thesis. A thesis is a public document, and can be viewed through the library at the University of Canterbury.

If you want, you can have a copy of the publications that arise from this research. Please be aware that a substantial delay may occur between data collection and completing the final report. Alternatively, you can choose to have findings discussed with you personally by the lead investigator.

20. Where can you go for more information, or to raise concerns or complaints?

If you have any questions, concerns or complaints about the study at any stage, please contact:

Sarah Davies, Ph.D. candidate
(03) 364 2307
sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee, Senior researcher
(03) 364 2014
maggie-lee.huckabee@canterbury.ac.nz

If you want to talk to someone who isn't involved with the study, contact an independent health and disability advocate:

Phone: 0800 555 050
Email: advocacy@hdc.org.nz

You can also contact the ethics committee that reviewed and approved this study:

Phone: 0800 4 ETHICS

Email: hdecs@moh.govt.nz

Participant Information Sheet

Study title:	**The relationship between oral bacteria, cough reflex sensitivity, and pneumonia**		
Locality:	**Southern**	Ethics committee ref.:	**13/STH/121**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

You are invited to take part in a research project about swallowing in patients with stroke. Taking part is your choice. If you don't want to take part, you don't have to give a reason. It won't change the care you receive. If you want to take part now, but change your mind later, you can pull out of the study at any time.

This information sheet will help you decide if you'd like to take part. It sets out why we are doing the study and what is involved. It also explains the risks and benefits, and what happens after the study. We will go through this with you and answer your questions. This will take about 5 minutes. You may also want to talk about the study with family, whānau, friends, or healthcare members. Feel free to do this.

If you want to take part, you will need to sign a consent form. You will be given a copy of this information sheet and consent form to keep.

This document is 5 pages long. Please make sure you have all the pages.

This study is being run by Sarah Davies with supervision from Dr Maggie-Lee Huckabee (University of Canterbury), Dr John Fink and Dr Geoffrey Tompkins (University of Otago). The University of Canterbury (UC) and the Canterbury District Health Board (CDHB) are collaborating in this study. This study has been approved by the UC Human Ethics Committee, the CDHB Ethics Committee, and The New Zealand Health and Disability Ethics Committee.

For more information, please contact the researchers during work hours. Their numbers are written at the end of this document.

If you need an interpreter, this can be arranged.

If you have any queries or concerns about your rights in this study, contact a Health and Disability Advocate:

South Island: 0800 377 766 or (03) 377 7501 in Christchurch.
Email: advocacy@hdc.org.nz

1. Why are we doing the study?

In some patients with stroke, the ability to carry out their usual oral care routine changes. They might also have a reduced cough reflex, and/or trouble swallowing. These patients are at risk of getting chest infections. We want to know whether there is a link between bacteria in the mouth, the cough reflex, and chest infections in people with stroke. The results from this study will help improve our care of stroke patients.

2. What is involved?

Taking part in this study will not change your care. Your care will be the same whether or not you enrol in this study.

If you agree to enrol in the study, the following will occur:

6. You will be asked to sign a consent form.
7. A researcher will take a sample of your saliva. She will do this by placing a small sponge under your tongue for 45 seconds. She will do this twice. These samples will be sent to the University of Otago Dental School for analysis.
8. The researcher will then test your cough reflex:
    a. A face mask will cover your nose and mouth. The face mask is attached to a device that turns liquid into mist, called a nebuliser.
    b. The nebuliser will turn small amounts of citric acid or saline into mist. You will be asked to quietly breathe the mist for 15 seconds.
    c. The mist may make you want to cough. Cough if you need to, but don't cough if you don't feel the need to.
    d. This test will be repeated between 3 and 16 times depending on your response.
9. This whole process will happen a further 2 times – once when you leave the acute stroke ward, and again in 30 days' time. The researcher may visit you in your home to do this.
10. The researcher will also check your medical file to see if you developed a chest infection. She will also phone your GP to check this.

3. What are the possible benefits and risks?

There is no benefit to you from doing this study.

There are no known adverse side effects of cough reflex testing. The test is done by a trained researcher who will monitor you for adverse signs.

4. What would happen if you were injured in the study?

If you were injured in this study, which is unlikely, you could apply for compensation from ACC just like if you were injured in an accident at work or home.

5. What are your rights?

Taking part in this study is your choice. If you don't want to take part, you don't have to give a reason. It won't affect your care. If you want to take part now, but change your mind later, you can pull out of the study at any time.

You have the right to see your personal information collected during this study at any time. If, during the study, new information about the risks and benefits come to light, you will be told of this.

Your medical information will be kept confidential by using a number instead of your name. All information will be stored in a locked cabinet at the Rose Centre for Stroke Recovery & Research at St George's Medical Centre. Electronic data will be stored on a password-protected computer at the Rose Centre.

6. What will happen after the study ends, or if you pull out?

The data we collect will be kept securely at the Rose Centre for 10 years. After this, it will be shredded under the supervision of either Sarah Davies or Maggie-Lee Huckabee.

The saliva samples will be analysed at the University of Otago Dental School and then destroyed by incineration.

Findings from this study will be prepared for publication in international journals. Results may be presented at national and/or international conferences. Findings will also form the basis of a doctoral thesis. A thesis is a public document, and can be viewed at the University of Canterbury library.

If you want, you can have a copy of the publications that arise from this research. Please be aware that a substantial delay may occur between data collection and completing the final report. You can also choose to have findings discussed with you personally by the lead researcher.

7. Where can you go for more information, or to raise concerns or complaints?

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(03) 364 2307
sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee, Senior researcher
(03) 364 2014
maggie-lee.huckabee@canterbury.ac.nz

If you would like to talk to someone from the CDHB Nga Ratonga (Maori Health Service), please contact:

Eru Waiti, Pouārahi Rōpū Ngā Rātonga Hauora (Māori Team Leader)
(03) 378 8797 ext 88797

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Phone: 0800 4 ETHICS

Email: hdecs@moh.govt.nz

Participant Consent Form

Study title:	**The relationship between oral bacteria, cough reflex sensitivity, and pneumonia**		
Locality:	**Southern**	Ethics committee ref.:	**13/STH/121**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

Declaration by participant:

I have read (or have had read to me in my first language) and I understand the Participant Information Sheet. I have had the opportunity to ask questions. I am satisfied with the answers I have received.

I freely agree to participate in this study.

I have been given a copy of the Participant Information Sheet and Consent Form to keep.

☐ I consent to the researcher contacting my GP and being provided with information about my health, as it relates to this study

☐ I would like the researcher to consult with my Iwi before collecting my saliva sample

☐ I consent to my saliva sample being disposed of using standard disposal methods at the end of the study

☐ I would like my saliva sample to be disposed of with appropriate karakia

☐ I would like to receive a copy of the results of this study

Participant's name: _____

Signature: _____ Date: _____

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant. I have answered their questions. I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ Date: _____

Information Sheet for Participants with Aphasia

Research into cough, bacteria, and chest infection in patients with stroke

Researchers:

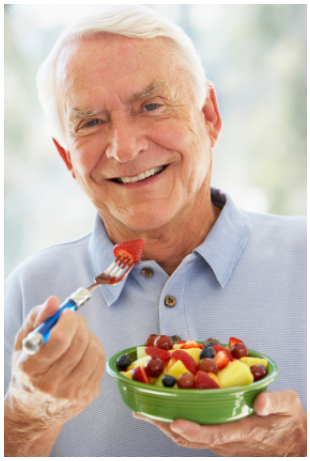
Sarah Davies
PhD student
University of Canterbury

Dr Maggie-Lee Huckabee
Senior researcher
University of Canterbury

Dr John Fink
Consultant neurologist
Canterbury District Health Board

Dr Geoffrey Tompkins
Senior researcher
University of Otago

We are researching **swallowing** in patients with **stroke**.



Taking part in this study is **your choice**.

my choice

You may **want to talk about the study** with other people, such as family, whānau, friends, or healthcare providers.

If you agree to take part in this study, you will be asked to **sign a consent form**.

9. Why are we doing the study?

In many patients with **stroke**, the **natural cough** may be affected.

Swallowing, and being able to **clean the teeth and mouth** may also be affected.

These patients may get a **chest infection**.

We want to **learn** if there is a link between **cough**, **bacteria in the mouth**, and **chest infection**.

This will help to improve the **care** of patients with **stroke**.

10. What would I have to do?

If you agree to take part in this study, you will need to **sign a consent form**.

A researcher will then take a **sample** of your **saliva**. She will put a **sponge** in your **mouth**, to soak up **saliva**.

You will then have a **cough** reflex **test**.

- A **face mask** will cover your nose and mouth.

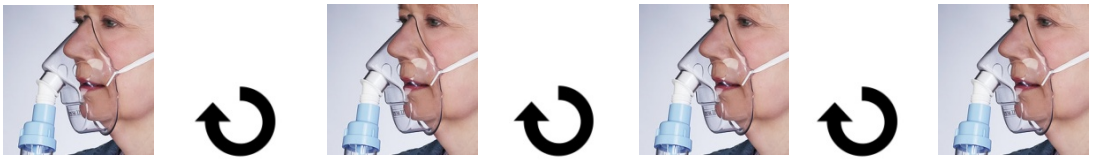
- The face mask is joined to a **nebuliser**.

- The nebuliser will turn **citric acid** into **mist**.

- You will be asked to **breathe** in the **mist** for 15 seconds.

- The mist may make you want to cough. **Cough** if you **need** to.

- We will do this test between **3** – **16** times.



The researcher will **come back** when you **leave** the acute stroke **ward**, and do these tests again.



The researcher will come and **visit you** again in **30 days**, and do these tests for the last time.


You will not need to do anything else.

11. What happens next?

The **researcher** will look at your **medical record** to see if you had a **chest infection**

The **researcher** may also phone your **GP**.

12. What are the risks and benefits?

This will **help researchers** learn more about swallowing treatment.

This may **help people** who have a **stroke**.

There is **no benefit** to you.

There are **no risks** to you.

Being involved in this study will **not change your treatment**.

You will receive the **same treatment** whether you take part in this study, or not.

This is **not therapy**.

13. What would happen if I was injured in the study?

If you were **injured** in this study, which is **unlikely**, you could apply for compensation from **ACC** just like if you were injured in an accident at work or at home.

14. What are my rights?

Taking part in this study is **your choice**.

If you **do not want to** take part, you do not have to give a reason**.**
It won't change your care.

If you want to take part now, but **change your mind** later, you can **stop** at any time.

You have the right to **see your personal information** at any time.

If there is **new information** about the risks and benefits, we will **tell you**.

15. What will happen to my personal information?

All information is **confidential**. We will use a number instead of your name.

The **saliva** samples will be kept securely in a **freezer** at the Rose Centre.

They will then be **sent** to The **University of Otago** Dental School for **testing**.

UNIVERSITY of OTAGO

SAPERE AUDE

Te Whare Wānanga o Otāgo

We will **not** keep the saliva samples. After testing, they will be **destroyed** by fire. If you like, a **karakia** or **prayer** can be said.

The information will be kept on a **password**-protected **computer** at the Rose Centre.

At the end of this study, the information will be **kept safely** at the Rose Centre for **10 years**. Then it will be **destroyed**.

10 years →

Findings from this study may be **presented**:

- In scientific journals
- At scientific meetings
- In a doctoral thesis, which the **public** can read at the University of Canterbury **library**

If you want, **you can have a copy** of the findings. This may take some time to prepare.

If you want, the main researcher will tell you about the findings.

16. Where can I go for more information about the study?

If you have any **questions** about the study at any stage, please contact:

Sarah Davies

Ph.D. candidate

(03) 364 2307

sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee

Senior researcher

(03) 364 2014

maggie-lee.huckabee@canterbury.ac.nz

17. Where can I go to raise concerns or complaints about the study?

If you want to **talk** to someone who is not part of the study, you can contact a **Health and Disability Advocate**:

Phone:  0800 555 050

Email:  advocacy@hdc.org.nz

If you have any **queries** or **concerns** about your **rights** as a participant in this study, you can contact a **Health and Disability Advocate**:

 Christchurch: (03) 377 7501

 South Island: 0800 377 766

 Email: advocacy@hdc.org.nz

This research has been **approved** by the Human Ethics Committees of The University of Canterbury, The New Zealand Health and Disability Ethics Committee, and The Canterbury District Health Board.

You can **contact** the **ethics committee** that approved this study on:

Phone:  0800 4 ETHICS

Email:  hdecs@moh.govt.nz

If you would like to **talk** to someone from the **CDHB Nga Ratonga** (**Maori Health Service**), please contact:

Eru Waiti
Pouārahi Rōpū Ngā Rātonga Hauora
(**Māori Team Leader**)
Phone: 378 8797 ext. 88797

Consent Form for Participants with Aphasia

Study title:	**The relationship between oral bacteria, cough reflex sensitivity, and pneumonia.** ("Research into cough, bacteria, and chest infection in patients with stroke")		
Locality:	**Southern**	Ethics committee ref.:	**13/STH/121**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 378 6068**

I understand:

- I do not have to take part. It is **my choice.**

- I can **change my mind.**
- I can **ask questions at any time.**

- I can **stop** the research at **any time.**

- There is **no danger** in doing this research.

I understand what will happen:

- A researcher will take a **saliva** sample

- I will have a **cough** reflex **test**

- The researcher will visit me later **in hospital**, and do the **tests again**

- The researcher will visit me later **at my home**, and do the **tests again**

- The **researcher** will get information from **my medical records**.

I understand the benefits of this research:

- This will **help researchers** learn about swallowing care.
- This may **help people** who have a **stroke**.
- This will **not** benefit me.
- This is **not therapy**.

I understand that my personal information:

- Will be kept **safely**.
- Will be **confidential**.
- Will be destroyed after **10 years**.

I understand that my saliva samples:

- Will be **destroyed** by fire immediately after the research is done.

☐ I **understand** this research.

☐ I have had the chance to **ask questions** and I am happy with the answers.

☐ I **agree** to participate in this study.

☐ I **agree** to the **researcher** talking to my **GP** and getting **information** about my **health**.

☐ I would like the **researcher** to **talk** to my **Iwi** before collecting my **saliva sample**.

☐ I agree to have my **saliva sample destroyed** at the end of the study

☐ I would like my saliva sample to be destroyed with a **karakia**

☐ I have been given a **copy** of the Participant **Information** Sheet and Consent Form to keep.

☐ I would like to see a **copy of the results** of this study

Name:

Signature: Date:

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it. I believe that the participant understands the study and has given informed consent to participate.

Researcher's name:

Signature: Date:

STATEMENT BY RELATIVE/FRIEND/WHANAU

Lay title:	The relationship between oral bacteria, cough reflex sensitivity, and pneumonia
Principal investigator:	Sarah Davies
Participant's name:	

I have read and I understand the information sheet dated 21.08.13 for people taking part in the study designed to evaluate bacteria in the mouth, the body's cough reflex, and chest infections in patients who have had a stroke. I have had the opportunity to discuss this study. I am satisfied with the answers I have been given.

I believe that _____ (participant's name) would have chosen and consented to participate in this study if he/she had been able to understand the information that I have received and understood.

I understand that taking part in this study is voluntary and that my relative/friend may withdraw from the study at any time if he/she wishes. This will not affect his/her continuing health care.

I understand that his/her participation in this study is confidential and that no material which could identify him/her will be used in any reports on this study.

I know whom to contact if anything occurs which I think he/she would consider a reason to withdraw from the study.

This study has been given ethical approval by the Northern A Regional Health & Disability Ethics Committee. This means that the Committee may check at any time that the study is following appropriate ethical procedures.

I/my relative/friend would like a copy of the results of the study.	☐ Yes	☐ No
I believe my relative/friend would agree to his/her GP being informed of his/her participation in this study.	☐ Yes	☐ No

Signed:		Date:	
Printed name:			
Relationship to participant:			
Address for results:			

STATEMENT BY INDEPENDENT CLINICIAN

I confirm that participation in the study is not adverse to _____ *(participant)*'s interests.

Signed: _____ Date: _____

(Clinician)

Printed name: _____

STATEMENT BY PRINCIPAL INVESTIGATOR

I _____ *(name of investigator)* declare that this study is in the potential health interest of the group of patients of which _____ *(name of participant)* is a member and that participation in this study is not adverse to _____ *(name of participant)*'s interests.

I confirm that if the participant becomes competent to make an informed choice and give an informed consent, full information will be given to him/her as soon as possible, and his/her participation will be explained. If the participant makes an informed choice to continue in the study, written consent will be requested and if the participant does not wish to continue in the study, he/she will be withdrawn.

Signed: _____ Date: _____

(Principal Investigator)

(IF APPLICABLE AT A LATER STAGE)

I _____ *(participant)* having been fully informed about this study agree to continue taking part in it.

Signed:		Date:	

(Participant)

Participant Information Sheet

Study title:	**The relationship between oral bacteria, cough reflex sensitivity, and pneumonia**		
Locality:	**Southern**	Ethics committee ref.:	**13/STH/121**
Lead investigator:	**Sarah Davies, MSc**	Contact phone number:	**(03) 364 2307**

You are invited to take part in a research project about swallowing in patients with stroke. Taking part is your choice. If you don't want to take part, you don't have to give a reason. It won't change the care you receive. If you want to take part now, but change your mind later, you can pull out of the study at any time.

This information sheet will help you decide if you'd like to take part. It sets out why we are doing the study and what is involved. It also explains the risks and benefits, and what happens after the study. We will go through this with you and answer your questions. This will take about 5 minutes. You may also want to talk about the study with family, whānau, friends, or healthcare members. Feel free to do this.

If you want to take part, you will need to sign a consent form. You will be given a copy of this information sheet and consent form to keep.

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This study is being run by Sarah Davies with supervision from Dr Maggie-Lee Huckabee (University of Canterbury), Dr John Fink and Dr Geoffrey Tompkins (University of Otago). The University of Canterbury (UC) and the Canterbury District Health Board (CDHB) are collaborating in this study. This study has been approved by the UC Human Ethics Committee, the CDHB Ethics Committee, and The New Zealand Health and Disability Ethics Committee.

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8. Why are we doing the study?

In some patients with stroke, the ability to carry out their usual oral care routine changes. They might also have a reduced cough reflex, and/or trouble swallowing. These patients are at risk of getting chest infections. We want to know whether there is a link between bacteria in the mouth, the cough reflex, and chest infections in people with stroke. The results from this study will help improve our care of stroke patients.

9. What is involved?

Taking part in this study will not change your care. Your care will be the same whether or not you enrol in this study.

If you agree to enrol in the study, the following will occur:

1. You will be asked to sign a consent form.
2. A researcher will take a sample of your saliva. She will do this by placing a small sponge under your tongue for 45 seconds. She will do this twice. These samples will be sent to the University of Otago Dental School for analysis.
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4. This whole process will happen a further 2 times – once when you leave the acute stroke ward, and again in 30 days' time. The researcher may visit you in your home to do this.
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10. What are the possible benefits and risks?

There is no benefit to you from doing this study.

There are no known adverse side effects of cough reflex testing. The test is done by a trained researcher who will monitor you for adverse signs.

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If you were injured in this study, which is unlikely, you could apply for compensation from ACC just like if you were injured in an accident at work or home.

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13. What will happen after the study ends, or if you pull out?

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sarah.davies@pg.canterbury.ac.nz

Maggie-Lee Huckabee, Senior researcher
(03) 364 2014
maggie-lee.huckabee@canterbury.ac.nz

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